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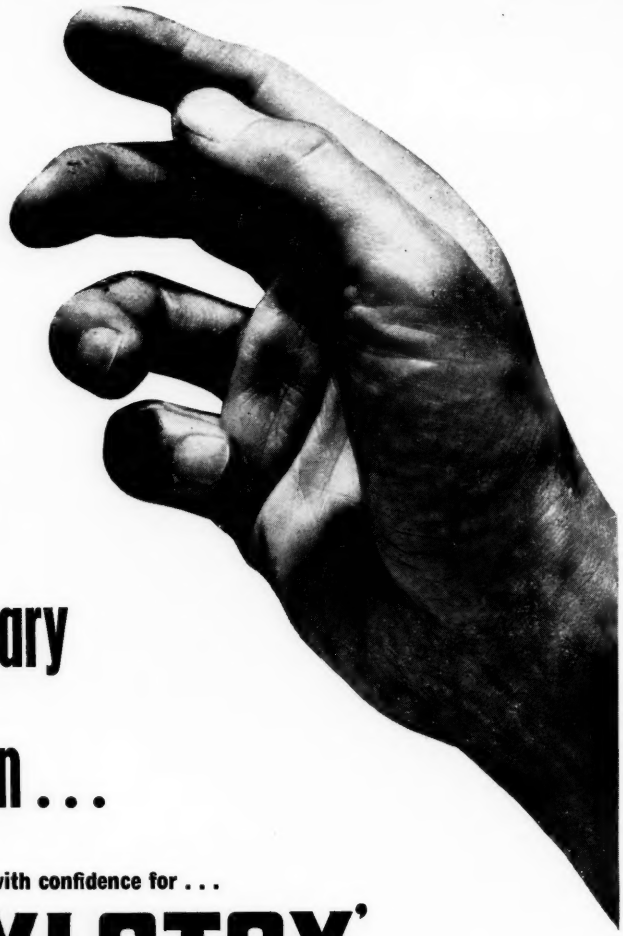

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THE BRITISH VETERINARY JOURNAL

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EDITORIAL

BREEDING PROBLEMS

THE new regulation of the Kennel Club, which will come into operation on January 1st, 1959, prohibits the exhibition of dogs which are not entire. This is a salutary measure which has been long overdue. Its repercussions must be for the eventual good of the canine world. No doubt one of the main reasons for the promulgation of this regulation is the fact that the developmental errors which lead to monorchidism or crytorchidism are amongst those which are known to be transmitted to the progeny. In the individual animal the defect may be resolved by surgical or other means and with apparent success as far as that particular animal is concerned and the latter may pass muster as a normal entire dog. The physical defect may be rectified but the inherent potential for the transmission of the abnormality will remain. Dogs that are abnormal in this way often show a fertility rate which is very low or it may even be a negative one. This is one of nature's ways of controlling or eliminating the effects of her own mistakes. Nature is wise also in another way for by her rule an organ which is abnormal or misplaced or misused is more liable to disease than is one that is normal. A sexual organ or gland which is affected by disease may become impotent and then the question of transmission does not arise. The so-called hereditary diseases and developmental abnormalities have been considered of prime importance by breeders of all kinds of stock and particularly by those who breed horses. Government committees and commissions have deliberated on these problems and certain defects such as cataract, ringbone, etc., have been designated as being of an hereditary nature and specifically mentioned in legislative measures enacted for the improvement of horse breeding. In recent years doubts have been expressed whether there is any real truth in some of the theories which have been accepted in the past. For instance, is there firm proof that acquired abnormalities of the skeleton such as ringbone or spavin or

opaque areas in the crystaline lens devolve from the machinations of some aberrant gene in the victim's progenitors. Experience would seem to support the contention that circulatory defects and environmental factors associated with defective nutrition and trauma are more culpable. There is some evidence that functional physiological irregularities seem to be repeated in certain families or blood lines. This would appear to be the case in regard to those structures over which the autonomic system holds a measure of control, i.e. the circulatory, respiratory, alimentary systems. The endocrine system which controls the activities of the sex organs and is responsible in part for fertility undoubtedly comes under strong suspicion. The endocrine control of these structures is so delicately balanced and controlled with a fine trigger-like touch that it may become capricious and thwart the objective for which they were designed. The health of the endocrine system cannot be judged by the effect upon one organ such as the testicle or ovary. The actions of the internal secretory glands are integrated and correlated to such a degree that faults in one portend trouble in others. When they work agreeably and in cohesion the metabolism of the body responds in normal healthy function. If one section be stimulated to undue effort repercussions may occur in other parts. The present scourge of infertility in dairy cows is an example of this law. Milk production has been enhanced to a level far above that which nature designed. It is not surprising that when the physiological function of the mammary gland has been so rudely upset the action of other parts, such as the ovaries, may register a change of action also. One may be able to rectify abnormal action in the ovaries by surgical or medicinal means. One may be able to make an infertile cow fertile but it must be remembered that endocrine dysfunction can be transmitted and for the good of the race it might be the best policy to eliminate those cows which cannot naturally stand the strain and think of the future rather than of the present. In many cases a gun can bring greater benefit to the race than a hypodermic syringe.

STUDIES ON INTERVERTEBRAL DISC PROTRUSION IN THE DOG

By L. C. VAUGHAN

Department of Surgery, Royal Veterinary College, Streatley, Berks.

2. DIAGNOSIS OF THE DISEASE

THE etiology and pathogenesis of intervertebral disc protrusion in the dog formed the subject matter of a previous paper by the present author (Vaughan, 1958). In the present article, consideration is given to the incidence of the disease, its clinical manifestations and the use of radiography in the accurate location of the lesion.

(a) Incidence

The *breed incidence* in relation to the regional distribution of the protrusion and the *sex incidence* in the 65 dogs investigated are shown in Table No. I. The outstanding finding is the high frequency in the Dachshund, the large majority of which had thoraco-lumbar protrusions. In contrast there was a predominance of cervical cases in the Spaniels. No significance can be attached to the slight over-all preponderance of females over males.

TABLE I

BREED	NUMBER OF DOGS	REGIONAL DISTRIBUTION		SEX	
		CERVICAL	THORACO-LUMBAR	MALE	FEMALE
1. Dachshund	34	3	31	13	21
2. Cocker Spaniel	9	6	3	4	5
3. Springer Spaniel	1	1	-	-	1
4. Crossbred Spaniel	3	1	2	3	-
5. Pekingese	8	2	6	2	6
6. Corgi	3	1	2	2	1
7. Sealyham	2	-	2	1	1
8. Boxer	1	1	-	1	-
9. Collie	1	-	1	1	-
10. French Bulldog	1	1	-	1	-
11. Foxhound	1	-	1	1	-
12. Terrier	1	-	1	1	-
TOTALS	65	16	49	30	35

The *age incidence* of affected dogs is shown in the graph (Table III) where it can be seen that ages ranged between 2½ and 10 years. The most noticeable feature is the high frequency of disc protrusion in five and six-year-old dogs.

(b) Signs and Symptoms

Irrespective of the site of the protrusion, signs and symptoms generally fall into two categories. In the one the predominant sign is regional pain. In the other there

is locomotor disturbance which may or may not be associated with regional pain. Before discussing signs and symptoms in detail it might not be out of place here to analyse the 65 disc protrusion cases investigated (Table No. II).

The significant factor which emerges from this simple analysis is the relative frequency with which partial or complete loss of motor power of the hindquarters is associated with thoraco-lumbar disc protrusion. It is obvious that this division of cases into those which exhibit pain only and those with locomotor dysfunction is purely arbitrary since the painful stage may be a precursor of some form of paralysis.

TABLE II

	Cervical disc protrusion cases	Thoraco-lumbar disc protrusion cases
Regional pain	10	6
Quadriplegia	5	-
Partial quadriplegia	1	-
Incoordination & partial paraplegia	-	10
Paraplegia	-	33
Total	16	49

Cervical Protrusion

Cervical pain is first noticed when the dog resents being patted or handled about the neck. The dog which was normally placid becomes very apprehensive and difficult to handle. There is seldom a history of antecedent injury. The dog is dejected, disinclined to move, reluctant to go up and down stairs, and refuses food placed at ground level. When the pain is acute the back is arched, the cervical muscles are tense and hard, and the head and neck are held in rigid extension (see Fig. 1; Vaughan, 1958). Any movement is accompanied by cries of pain. Palpation of the head and neck causes pain, which is pronounced particularly when the head is raised and depressed manually. The dog may take several minutes to adopt or get up from the brisket sitting position. In some cases knuckling of the carpus in one fore leg, a posture characteristic of radial paralysis may be observed. This symptom is usually temporary and disappears when the dog walks, but the reflexes in the affected limb are depressed.

Locomotor disturbance commences suddenly and takes the form of a partial or complete quadriplegia. It the latter case the dog lies on its side and is unable

to move from that position. The muscles of all four limbs are hypotonic and the spinal reflexes are absent (see Fig. 3; Vaughan, 1958). Palpation of the head and neck causes pain. If its head is supported the dog may be able to feed and drink. The dog cannot defæcate and the urine is retained. If the latter is not relieved a retention cystitis may develop. In partial quadriplegia there is muscle tone in all four limbs but the dog cannot stand unaided (Figs. 1 and 2). There is no loss of bowel or bladder control.

Thoraco-lumbar Protrusion

The condition commences with a sudden change in the temperament and habit of the dog. It becomes irritable, disinclined to move, and flinches when its back is stroked or patted. The hinds legs are moved stiffly, especially early in the day. The dog has difficulty in going up and down stairs and refuses to jump on to or off its own chair. These initial symptoms are accompanied or rapidly followed by pain which may vary in severity and may be intermittent. In some instances the dog screams when it moves or if it be touched almost anywhere about the body, and for this reason it is extremely difficult to make an examination. In others, pain is regional or may be localised to pressure on one particular vertebra. The lumbar and abdominal muscles are tense and hard. Often the spinal reflexes in the hind legs are exaggerated. Often a kyphosis at the level of the thoraco-lumbar junction is noted. Since the contraction of the abdominal muscles gives rise to pain the dog may become constipated and the urine may be retained for long periods. Appetite is maintained except during periods of acute pain. As a rule, this condition remains static but in some instances it progresses through ataxia to partial or complete paraplegia.

Inco-ordination or paresis is manifested by a weakness of one or both hind limbs and an abnormal gait. Ataxia is most pronounced when the dog tries to turn sharply or jump, and, in the male, when it raises its leg to urinate. Sometimes these symptoms are accompanied by pain which is most evident when the spine, in the vicinity of the protrusion, is palpated. Spinal reflexes are slightly depressed in the affected limbs. Urination and defæcation are normal. Over a period of several days the condition may progress to paraplegia.

Paraplegia usually develops suddenly and without previous history. In the morning the dog may be found to be paralysed although it had been quite well on the previous evening. In the majority of cases the paraplegia is of the flaccid type. The hind limbs are limp and they are dragged behind the body. The abdominal muscles are relaxed. Spinal reflexes are either depressed or absent from both hind legs. The tail does not respond to pin prick. Retention of urine and fæces is usual but occasionally there is urinary incontinence. The male dog exhibits priapism and passes urine in squirts when the bladder is evacuated manually. If the paraplegia is spastic the hind legs are stiff and they are extended forwards under or to one side of the body and the dog drags itself on its buttocks. In this form of paralysis it is not unusual for the dog to exhibit some degree of pain. Spinal reflexes are exaggerated. Urine and fæces are retained. Owing to the absence of subjective symptoms in animals it is often difficult to determine the precise area of the body which is desensitised by the compression of the cord. Individual response to sensory stimulation is extremely variable. Because the posterior spinal nerves

leave the neural canal some distance behind their point of origin the actual site of compression is one or two vertebra ahead of the sensory demarcation line.

In a small number of cases (four in the present investigation), acute flaccid paraplegia is followed in a few days by ascending cord symptoms. The dog loses the use of its fore legs, becomes moribund and dies as a result of respiratory failure.

Atrophy of thigh and lumbar muscles, excoriation of the dorsal aspect of the hind feet, decubital ulcers on the buttocks and varying degrees of cystitis occur as complications of chronic cases. The reflexes may improve and the tail react to sensory and motor stimuli, but little prognostic significance can be attached to these findings.

(c) Methods of Diagnosis

A consideration of the clinical manifestations, together with the history and breed susceptibility, may allow a tentative diagnosis of disc protrusion to be made. The particular signs displayed often indicate the region of the protrusion. In order to confirm the diagnosis and indicate the exact site of the lesion X-ray examination is necessary. This is essential where surgical treatment is contemplated.

Radiographical Examination—Without a Constrast Medium

As the lesions are small the spine must be immobilised in order to secure good definition. The positioning of the subject is very important also. Best results follow the use of a narcotic or an anæsthetic. Lateral views of the spine should be taken, in two or more sections, according to the size of dog. In this way an almost right-angle view can be obtained of each intervertebral disc and superimposition is minimal.

The significance of the radiographic lesions which may be demonstrated in the diagnosis of disc protrusion requires some comment.

1. *Calcification of the intervertebral disc*

Disc calcification is a common finding in the spines of dogs with disc protrusion but is also seen in apparently normal dogs especially those breeds which are susceptible to disc protrusion (Fig. 3). Calcification is an expression of degeneration and usually it is localised to the nucleus pulposus. There is no direct relationship between calcification and protrusion since relatively few calcified discs actually protrude into the spinal canal. Any of the 26 intervertebral discs may be calcified and the number varies considerably, as many as 10 having been found in one patient.

2. *Narrowing of the intervertebral disc*

Normally the lumbar discs are wider than the thoracic and this must be taken into consideration when comparing adjacent discs in the transition zone between these two regions. As a general rule a disc must be judged by comparison with those immediately anterior and posterior. Narrowing of a disc indicates a loss of tissue which may be the result of degeneration or the escape of nuclear material in the form of protrusion. One might reasonably expect a narrowing of the disc to occur following extrusion of its nucleus but this does not happen in all cases. Conversely it has been found that narrowing is not concrete evidence of protrusion.

3. *Calcification of the dorsal protrusion*

Small specks or complete cones of opacity seen immediately dorsal to the disc

PLATE I

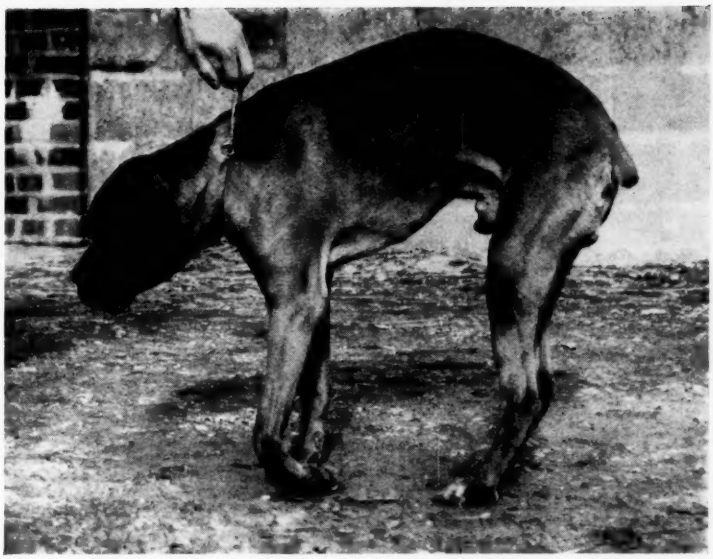


Fig. 1
3-year-old Boxer dog. Protrusion of 2/3 Cervical disc. Note flexion of left carpus. Able to stand when supported by neck chain.

FIG. 2
As in Fig. 1. Collapse of fore limbs when support was removed.

(Article by L. C. Vaughan, on page 203)

PLATE II

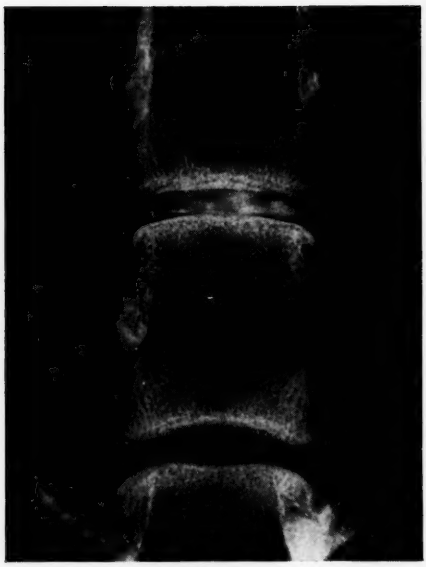


FIG. 3

Dorso-ventral radiograph, taken post-mortem, of a segment of spine from a 15-year-old Pekingese which was not showing symptoms. Note calcification of 5/6 Lumbar disc.

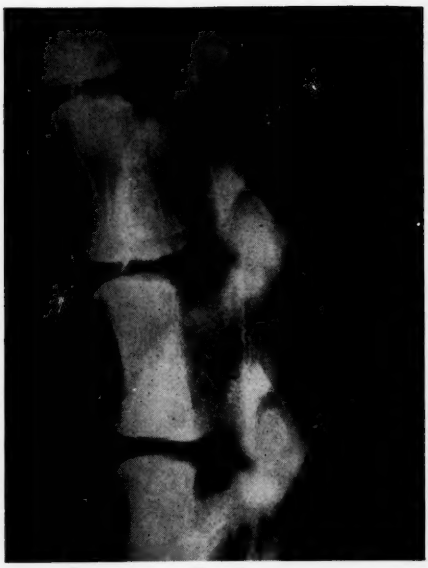


FIG. 4

Lateral radiograph showing calcified protrusion of 1/2 Lumbar disc in a 5-year-old Foxhound which was paraplegic.

FIG. 5

Myelograph of dog shown in Figs. 1 and 2. Lipiodol obstructed just anterior to the 2/3 Cervical disc indicating protrusion (confirmed at autopsy.)

FIG. 6

Myelograph of a 5½ years old Spaniel, with posterior paresis. Calcified protrusion of 4/5 Lumbar disc obstructing flow of Lipiodol.

(*Article by L. C. Vaughan, on page 203*)

and in line with the intervertebral foramen are positive evidence of a protrusion (Fig. 4). The disc itself may be calcified and/or narrow.

4. *Osteophytes*

Osteophytes on the ventral aspect of the thoracic and lumbar vertebræ may be observed quite frequently. They are of no significance as far as dorsal disc protrusion is concerned.

The value of radiography without the use of a contrast medium is summarised as follows:—

Of 62 protrusions found in the 31 dogs autopsied (one of the 32 dogs autopsied was not radiographed), 23 (37 per cent) were detected because calcification had rendered them radiopaque. An analysis of these 23 radiopaque protrusions revealed that in 12 there was calcification of the protrusion only; in three there was calcification of the disc also. In five there was narrowing of the disc in addition to calcification and in the remaining three the discs were narrowed only.

It is obvious that this technique has its limitations and if an accurate diagnosis of disc protrusion is to be made a myelographic study is essential.

Radiographical Examination—With a Contrast Medium (*Myelography*)

Myelography entails the introduction of a radiopaque medium into the spinal subarachnoid space so that the spinal cord may be outlined radiographically. The medium used by the author was Lipiodol Lafay, an iodised oil containing 40 per cent iodine. Its absorbability is practically nil and it has an irritant effect on the spinal meninges with a temporary increase in the cell count in the cerebrospinal fluid (Brook, 1936). It does afford good visual definition. It is injected immediately after the initial radiographic examination. The patient is given a general anæsthetic which must last for at least two hours in order to give time for the gravitation of the radiopaque material. Pentobarbitone sodium is the anæsthetic agent which has been employed.

Using the technique described by Brook (1936), the spinal subarachnoid space is entered at the occipito-atlantal joint. The needles used are of standard calibre (19 B.W.G.) and they vary in length (1 in. to 3 in.) depending on the size of the dog. Usually cerebrospinal fluid flows back along the needle but occasionally it is necessary to withdraw the fluid with a syringe, probably because the needle has become blocked with tissue during its insertion. The amount of fluid removed approximates in amount to the quantity of Lipiodol to be injected (1-4 c.c.), which in turn is dependent on the size of the dog and the suspected level of the protrusion. If during the insertion of the needle the dog displays spasm or tremor, the needle must be withdrawn and redirected to less depth. If blood escapes it is advisable to withdraw the needle completely and reinsert a clean one. The Lipiodol, previously warmed, is injected slowly. The dog is placed on its back immediately with its head uppermost on the radiographic table which is tilted at an angle of 30°-45°. The time taken for the radiopaque oil to reach the arachnoid cul-se-sac in the normal dog has been found to be approximately 90-110 minutes. This is in accordance with the average time of 108 minutes recorded by Brook (1936). The first radiograph is taken after 1½ hours if the signs suggest a thoraco-lumbar protrusion and, if necessary, further exposures are made at half-hourly intervals until the further

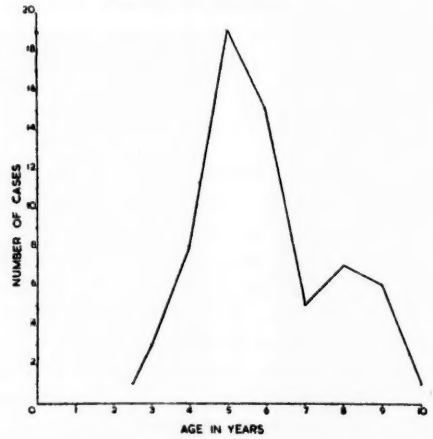
gravitation of Lipiodol is impeded. If the protrusion is cervical, a radiograph taken 30 minutes after injection is usually adequate. The degree of obliteration of the subarachnoid space depends on the size of the protrusion and its interference with the spinal cord. In consequence, the arrest of the flow of oil may be complete or partial (Figs. 5 and 6). It is found invariably that in those cases where the oil does eventually negotiate the obstruction there is a " piling up " anteriorly and a marked attenuation of flow posterior to the protrusion. If the obstruction be complete it is obvious that any protrusion caudal to that lesion will remain undetected. If the obstruction is partial then a second " piling up " occurs.

If myelography is performed too soon after the onset of symptoms, especially in paraplegics, the Lipiodol is held up at a higher level than the site of protrusion. Probably this is because of the œdema and vascular extravasation which occur in the canal at the time of protrusion. For instance, in one dog the myelograph taken on the first day after paraplegia showed an obstruction at the 7 Thoracic vertebra. Eight days later the dog was again anæsthetised, placed on the inclined table and the Lipiodol descended to the 12 Thoracic vertebra indicating a protrusion of the 12/13 Thoracic disc, which was confirmed at autopsy. For this reason, myelography ought to be delayed for five to seven days after the onset of symptoms.

Myelography was performed on 34 dogs with disc protrusion. Twenty-four

TABLE III

AGE-INCIDENCE GRAPH



were eventually destroyed and 23 autopsied. In 21 of these the protrusion has been located accurately. In three instances, for some obscure reason, possibly adhesion of oil or bad positioning of the dog on the table, the Lipiodol did not reach the site of protrusion although ample time was given for it to do so.

Removal of the Lipiodol after myelography has not been practised. In ten dogs which recovered after conservative or surgical treatment there has been no untoward sequel attributable to its retention in the subarachnoid space even though radio-

graphs taken months later have revealed that globules of oil were still present in the spinal canal and in the skull. It should be remembered that iodised oils are irritant to the tissues and they might well be responsible for producing inflammatory changes in the pia mater even though these may be only transient.

Discussion

A tentative diagnosis of disc protrusion may be made from the history and the clinical examination of the dog, and particularly if the patient is of a susceptible breed. It has been shown that by radiography of the spine without the aid of a contrast medium it is possible to confirm the diagnosis only in a comparatively small percentage of cases since only those protrusions which are calcified will be demonstrable. Simple radiographic procedure can be very valuable. It enables one to discount other conditions which may produce signs and symptoms similar to those associated with disc protrusion, such as spinal fracture and dislocation. After some experience considerable accuracy in the diagnosis and, indeed, the exact location of the protrusion may be reached by using the technique of myelography. It has to be realised that this technique is not without slight risk both at the time of the injection into the subarachnoid space and also by virtue of the irritant effect the radiopaque substance may have on the spinal meninges subsequently. When surgical treatment is contemplated it is essential that the precise site of the protrusion be known. It is primarily for this reason that myelographic contrast techniques are employed.

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DISEASES OF SHEEP IN GHANA (GOLD COAST)

By N. C. FRY, M.R.C.V.S.
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Introduction

LIVESTOCK production in the Gold Coast (now known as Ghana) is important because of the lack of some requirements in the diet of many inhabitants. In 1939 the Committee on Nutrition for the Colonial Empire reported as a result of a survey in Ghana that "broadly speaking, the diet is deficient in those foodstuffs which provide fat, good protein, vitamins and mineral matter. . . . The protein content is generally low". The problem of inadequate dietary protein had already been appreciated, and as early as 1920 the Government Veterinary Department had set up a Livestock Section with, amongst other objects, the aim of "establishing model livestock farms and the study of all questions relating to stock breeding and the improvement of local breeds". (Beal, 1921.) However, due to the presence of tsetse fly over large areas of the country and the corresponding incidence of trypanosomiasis, livestock production has been confined mainly to the relatively fly-free

areas in the northern region. Distances between production areas and markets in the south are great, and since many of the cattle cover this distance " on the hoof ", quality at the destination is poor and the prices are high.

For many years livestock production and improvement has been carried out by the Government Animal Health Department (formerly the Veterinary Department) on the coastal savannah plain (Fig. 1). The plain is largely free from tsetse flies but the carrying capacity of the vegetation is low, and during the dry season, from November to May, water is in short supply.

When the University College of the Gold Coast was established in 1948 and the Agricultural Research Station of the Faculty of Agriculture was opened on the coastal plain in 1953, it became possible to conduct further experiments on the improvement of livestock in general and sheep in particular—an aspect which, in the past, has received little attention.

In under-developed countries, veterinary surgeons play a vital part in regard to the introduction and improvement of livestock. This paper deals briefly with the disease problems encountered in native and exotic sheep at the Research Station.

Animals

Some local dwarf forest sheep were purchased in 1955 (Fig. 2). After a period of acclimatisation they were divided into three flocks and mated respectively with Blackhead Persian fat-rumped rams, U.K. Wiltshire Horn rams and Long-legged Sudan sheep (Fig. 3) purchased from the Haute Volta region of French West Africa.

Disease Survey

The flocks have been a source of interest from the disease point of view. Isolated occurrences of Anthrax, Redwater and Tetanus occurred, but serious outbreaks of other diseases have caused much more concern.

1. *Endo-parasites*

Infestation with endo-parasites has given rise to serious loss. Both tapeworms and roundsworms increased rapidly during 1955, and the losses of sheep during the rainy season in 1956 were heavy. In all probability this was due to the practice of confining the sheep in pasture areas of 10 to 15 acres—a break with the traditional methods of the country where semi-nomadic grazing is practiced. Worm egg counts of around 50,000 eggs per gram were noted frequently, and in one instance, in December 1955, 109,450 e.p.g. was recorded. It is probable that the large burdens were due to the constant reinfection of animals from the pasture.

Coccidia. In 1955 it was found that most of the sheep were affected by *Coccidia*, and at least two species were identified (this seems to be the normal state of affairs both in cattle and sheep in tropical Africa (Fiennes, 1956).). The adult sheep showed few clinical symptoms, but the lambs became weak, and showed inappetence, loss of condition, diarrhœa and the fæces were blood-stained.

Roundworms. Very heavy infestations have been encountered, particularly of *Hæmonchus contortus*, and there is little doubt that this was responsible for the high egg-count. Parasitic gastro-enteritis has been recognised as the chief cause of mortality amongst sheep—Beal refers to it in 1928 as " the curse of sheep and goat breeding in the Gold Coast "—and before the introduction of routine dosing

with Phenothiazine and Nicotine, Copper Sulphate and Arsenic (N.C.A.), infestation was very heavy amongst the lambs in particular, with bottle-jaw as a normal symptom. Outgrazing of flocks, associated with increased dosing and dosages has resulted in the general control of roundworms. Lambs appear to remain unthrifty for many months after the initial set-back.

Tapeworms. Very many cases of severe tapeworm infestation occurred in 1955 and 1956, chiefly due to *Moniezia expansa.* Many deaths amongst lambs occurred, due not only to loss of condition, diarrhœa and exhaustion, but in a considerable number of cases to physicial impaction of the intestine. Dosing with N.C.A. was not effective, and Ex. Felis Mas, although efficient, proved exceedingly expensive. Control has now been attained by the use of Lead Arsenate at four-weekly intervals. *Moniezia* continues as an ever-present source of potential trouble due to the number of *oribatid* mites which act as intermediate hosts.

2. *Ecto-parasites*

Sheep become infested, and rapidly, with ticks if dipping is not done at regular intervals; many species are present, notably *Amblyomma.*, the transmitting agent of Heartwater (Alexander, 1931). The chief biting flies are *tabanids,* which are numerous and troublesome. Infestation of sheep with Nasal Myiasis has been a source of worry for a long time in the Gold Coast—and in 1929 Fulton found that over 90 per cent of sheep and goats inspected were affected. Larvæ indistinguishable from *Œstrus ovis* have been recovered at examination post-mortem from the nasal sinuses, and treatment of the flocks have now begun.

3. *Heartwater*

As stated above, *Amblyomma spp.* of ticks are found on the Research Station, and during the first year of introduction of sheep. These ticks gave rise to considerable losses. Heartwater has been reported regularly over many years in the Gold Coast Animal Health Department Annual Reports and it has been the cause of death of many of the sheep at Government stations. The peracute form (Alexander, 1931) has been observed in Ghana. The animal suddenly collapses and dies—but the acute type, with temperatures ranging up to 106° F., has been more common. After a few days the sheep walks in circles with an unsteady gait, and then it collapses in convulsions making galloping movements with its feet. No immunisation measures were attempted, but destruction of the vector by dipping in B.H.C. at intervals of not more than five days has reduced the incidence to a minimum.

4. *Blue-tongue*

Blue-tongue, an infectious disease characterised by hyperæmia of the mucous membranes of the mouth and nose, broke out amongst some of the newly imported Wiltshire sheep, killing two out of six of them. No cases were seen amongst the local sheep or those born on the Station. It is inferred that the disease is endemic (it was reported in 1933 by Stewart) and that immunity is developed at an early age by reason of a mild but unapparent attack. It is possible that a natural host may be present on the Plains since *Culicoides* have been found, and Du Toit (1944) has shown that captured wild flies of this species may be infected and carry the virus. All importations from overseas are now injected immediately on arrival with vaccine from Onderstepoort, and this has proved efficacious.

5. *Contagious Pustular Dermatitis*

Mild cases of Contagious Pastular Dermatitis occur amongst the local lambs, with lesions on the ears and nostrils—also in many instances amongst the F.1 and F.2 generations—but the imported Wiltshires, Persians and Sudans have suffered severely. Inoculation of susceptible sheep with an emulsion containing live virus from scabs of infected animals (linear scarification on the thigh) confirmed the diagnosis. The Orf was far more virulent than that encountered in Great Britain and in Australia (Belschner, 1951), and spreads from the mucous membrances of the lips and nostrils to the ears, limbs and genitalia. Laboured breathing from occlusion of the nostrils; complete inability to feed and consequent death from starvation; pus-discharging sores on all lower extremities and external genitalia have all been noted in various cases. In some instances treatment and vaccination proved quite ineffective, in slaughter of the most severely infected animals was the only answer. Local stock appear to gain a solid immunity following mild early infection. Thomas (1945) states that the disease may be an indication of debilitated health arising from a mineral deficiency, but observations have not confirmed that possibility.

6. *Foot-rot*

This has been a continual scourge amongst the sheep. Many of the locally purchased native sheep arrived with deformed feet, which is a predisposing factor. Many cases were atypical, showing as small punctures, which became septic and the horn was " under run "; pain was intense, and usually it seemed to be located at the bulb of the foot. If the feet have become softened, many cases can appear almost overnight—several can be confidently forecast after a heavy rainstorm. In some cases, normal methods of treatment have proved ineffective, but they have yielded to 10 per cent. tincture of Chloramphenicol. Foot abscesses have been seen, but treatment with Sulphamezathine internally and Sulphanilamide powder externally has given successful results. It is thought that Beveridge's claim (1941) that apparently recovered sheep often harbour small inconspicuous lesions in which the organism remains viable for many months may be the explanation for the sporadic outbreaks of foot-rot.

7. *Pneumonia*

Very heavy losses were experienced during the first year when the Station was being stocked. The first signs of the disease was diarrhœa or dysentery, and this advanced rapidly to pneumonia which was invariably fatal. Treatment with Sulphaguanidine was effective at the diarrhœa stage, but once pulmonary symptoms were established nothing was of any use. *Corynebacterium pyogenes* was obtained in pure culture from the livers of affected animals and it was supposed that this organism was the cause of the condition; pyogenes toxoid proved useful in the treatment of the infection. Some doubt was thrown on the validity of the diagnosis when about a year later some sheep (including one of the Wiltshire rams) died of pneumonia. In these cases lung sequestra were found which suggested the presence of Contagious Ovine Pleuropneumonia (chronic " lungers "). It is possible that this disease is implicated also, since it is known to be enzootic in West Africa (Stewart, 1931). No case of this type has been seen in the last twelve months.

8. *Photo-sensitisation*

Several suspected cases of Photo-sensitisation occurred in sheep and lambs shortly after the onset of the rains in July 1956. Whilst the majority of the cases occurred in Wiltshire/local cross lambs, isolated instances were noticed in Sudanese, Persian and local sheep. Lesions were typical, and were located on the ears and muzzle, and around the eyes. Pigmented areas were not affected. Measures were taken to protect the affected animals by placing them in shading buildings, but of twenty-five cases five died. Examination post-mortem revealed fatty degeneration of the liver and kidneys. In one case there was myocardial degeneration also.

Phenothiazine had been used as an anthelmintic on the sheep for a considerable time before these lesions occurred, but the possibility of primary photo-sensitisation due to this drug was discounted since sheep appear generally to be immune from all ill-effects (Swales, 1940). The liver damage which was observed on examination post-mortem suggests that the condition might have been of hepatic origin. The observed cases occurred with the fresh flush of pasture shortly after the onset of the rainy season, and thus is resembles Geeldikkop (Rimington and Quinn, 1937) since Tribulis spp. plants are present. There has been no recurrence, but liver damage of varying severity is still observed in the affected animals after their death from other causes.

9. *Trypanosomiasis*

A number of the native sheep imported into the Research Station were chronically or cryptically infected with trypanosomes, and a number of deaths have been attributed to this cause. Some of the imported Persian sheep have died from this cause also, indicating mechanical transmission of the trypanosomes, since the Station is in a tsetse fly-free area. *Stomoxys* and *Tabanidæ* are found on the Plains, and Hoare reported in 1936 that these species are probably concerned with outbreaks occurring in areas where tsetse is scarce. Routine Antryside protective treatment is carried out on all exotic sheep. The species of Trypanosome was not identified, but from observations *T. vivax* was probably the causal organism. This trypanosome has been responsible for many serious outbreaks in West Africa (Mettam, 1939; Stewart, 1935, 1938).

An atypical apoplectic form of trypanosomiasis were encountered, animals being found dead without showing any premonitory symptoms; diagnosis was often difficult, parasites being found only after prolonged searching of smears from a large number of tissues (Fiennes, 1956).

10. *Enterotoxæmia*

Several cases of Enterotoxæmia were identified by R. N. Fiennes (former Senior Lecturer in Animal Health). The sheep were adult, and death occurred suddenly at the end of the drought period after a heavy storm of rain. In some instances animals were found dead, in others a rapid transition occurred from slight sickness to death in convulsions. Findings at post-mortem examination were typical, including sub-, epi and endocardial hæmorrhages and patchy congestion of the mucous membrane of the small intestine and *Cl. welchii* were isolated, cultured and identified. No further cases have been observed, but enterotoxæmia is unpredictable in onset and incidence (Henning, 1955). The organism is present in the intestinal

tract of normal sheep (Bullen, 1952), and becomes lethal after the occurrence of a contributory or predisposing factor—it is thus interesting to record that signs were observed in two sheep which were being treated for unthriftiness with a high plane ration. This, after a long period of poor pasture during the dry season tends to confirm Bosworth and Glover's contention (1935) that a change of diet from low to high protein may serve as a contributory factor.

Discussion

An attempt has been made to list the chief causes of sheep mortality as encountered at the Agricultural Research Station on the Accra Plains. Simpson stated in 1949 that much research work on sheep diseases is needed before a sheep breeding industry can be developed in Ghana, and that view has been amply substantiated. One point that has been demonstrated repeatedly is that in cases of severe debilitation it is exceptionally difficult for the animal to regain condition.

Further research into sheep diseases, their causes and prevention, is in progress.

Summary

The main diseases of sheep encountered at the Agricultural Research Station of the University College of Ghana are listed; these include Heartwater, Blue-tongue, Contagious Purulent Dermatitis, Anthrax, Tetanus, Enterotoxæmia and worm infestation. Some account is given of preventive measures and treatment. An outbreak of Photosensitisation is described together with some observations on possible causation.

Acknowledgements

My thanks are due to Mr. R. N. T-W-Fiennes for his helpful comments in the preparation of this paper.

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PLATE I

FIG. 1

(x = site of Research Station.)

Description : Rainfall 25″ to 42″ p.a. concentrated between the months of March and July (major rains) and September and November (small rains).
Temperature : 85° average, fluctuating little.
Humidity : 79.6% average.

FIG. 2

FIG. 3

(*Article by N. C. Fry, on page 209*)

PLATE I

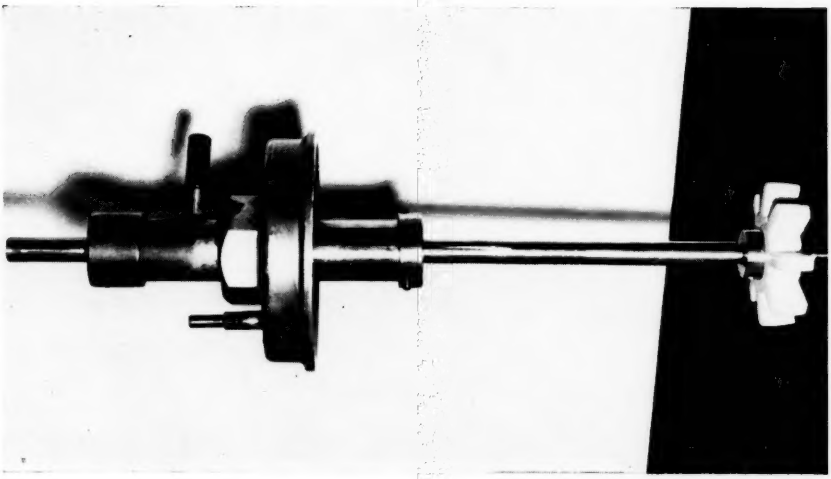

FIG. 2
The shaft and impeller assembly. The side-arm on the bearing housing is unnecessary.

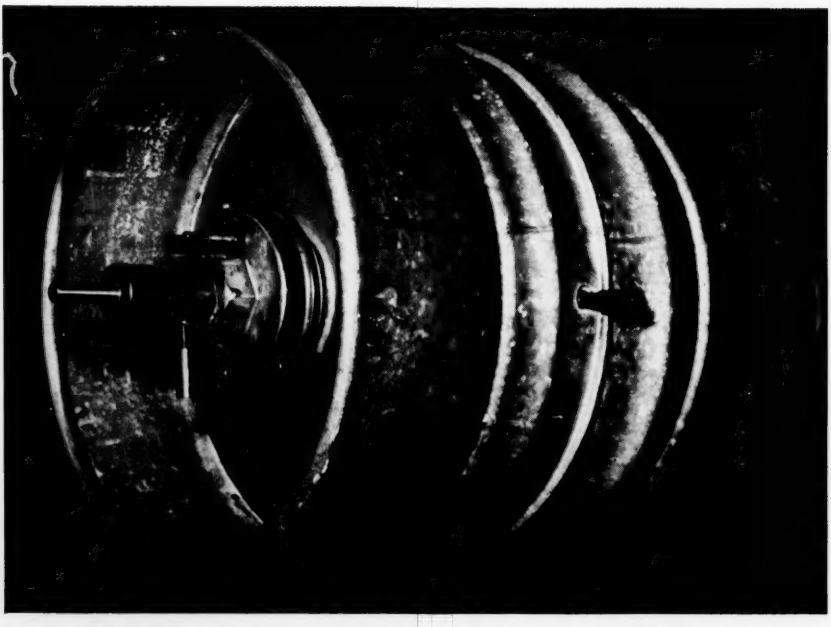

FIG. 1
General view of tank. The motor is mounted separately.

(Article by R. V. S. Bain and R. E. Jones, on page 215)

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THE PRODUCTION OF DENSE CULTURES OF PASTEURELLA MULTOCIDA

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Introduction

A SIMPLE method of obtaining pasteurella cultures of moderate density was described by Bain and Jones (1955). In recent years, there have been advances in the general methods of mass-cultivation of bacteria and these have been described by Finn (1954), Herbert, Elsworth and Telling (1956) and in Monograph XVII from the Istituto Superiore di Sanita of Rome (1954). Experiments incorporating these developments were conducted in our laboratory to secure good yields of *Pasteurella multocida* Type I (Roberts) for the production of vaccine against hæmorrhagic septicæmia of cattle.

Materials and Methods

Two aspects required examination, the type of apparatus and the culture medium. The apparatus had to be rugged in construction and free from operating troubles to suit laboratories in undeveloped countries; the medium had to be simple, cheap and able to be sterilised by heat. Two aeration systems were tried, the conventional sparger type and the vortex aeration (Chain, Paladino, Callow, Ugolini and van der Sluis, 1952).

Sparger Aeration

This is described briefly as it was not adopted for the final apparatus. The reaction vessel was a 10-litre pyrex bottle. Two holes were bored in the bottom for the reception of short filter candles which acted as air distributors. Frothing was controlled mechanically by rotating three swing-out stainless steel blades at 1,425 r.p.m about two inches above the level of the medium. The stainless steel shaft driving the blades entered the bottle through a twin ball-race assembly mounted in stainless steel tubing. The shaft was coupled flexibly to a ¼ h.p. constant-speed ball-bearing motor. Air entered the candles from a compressor via an air filter. Glass nipples were fused onto the bottle to permit entry of air, seed material and fresh medium and exist of air and culture. The reaction volume was maintained at five litres for continuous culture operation. The vessel was kept under slight positive pressure to prevent entry of contaminants.

FIG. 2 The shaft and impeller assembly. The side-arm on the bearing housing is unnecessary.

General view of tank. The motor is mounted separately.

Vortex Aeration

The principles adopted follow those of Chain *et al.* (*loc. cit.*).

A vessel was made from a 10-litre pyrex bottle, which had the advantage of making inspection easy. At a later date, vessels were made from 22 and 44-litre stainless steel containers mounted in galvanised iron casing (beer barrels). This particular kind of Australian barrel has at one end a central aperture of 9 cm. diameter closed by a threaded cap. On the latest version stainless steel nipples covered by small threaded caps are mounted on this central cap instead of in the fabric of the barrel except for the outlet nipple which is situated half-way up the vertical face of the vessel.

Aeration is effected by the action of an eight-bladed turbine impeller operating at a level one-third up from the bottom of the culture. The impeller is mounted on a stainless steel shaft (1.25 cm. diameter) which is rotated through a gland and bearing assembly at 950 r.p.m., the speed of a stock ¼ h.p. constant-speed electric motor. The impeller is one-sixth the diameter of the vessel and is machined from a " Teflon " or " Novabestos " block, or made up from stainless steel. The heavy shaft imparts enough rigidity to obviate the need for a bottom bearing. Filtered air is introduced through a nipple from a compressor or cylinder and it escapes from another nipple into an exit filter. A trap-bottle may have to be inserted on the air-exit line to catch condensate. A simple exit valve regulates the small positive pressure in the tank.

For operation under conditions in Sydney for periods of 24-48 hours at 37° C., it has not been necessary to use elaborate stuffing boxes or steam seals in the shaft assembly. Provided that sterilisation is effective to start with, there is not likely to be trouble from contaminants entering the tank during the brief period of growth.

For continuous operation, reservoirs of medium held in the same hot-room or in a waterbath, are connected to the culture vessel by rubber tubing which is sealed to the glass nipples with a glass-rubber cement. Glass transfusion drip counters are put in the lines to gauge the rate of flow.

Seeding the tank is effected through an expendable rubber diaphragm. The cap on one of the nipples is double. A small disc of sheet rubber cut from any convenient material is placed under the first cap. Under the second superimposed cap a pad which can be saturated with formaline is placed. A needle attached to a syringe or flask can be plunged quickly through the diaphragm for sowing the culture vessel. The volume of seeding culture is, on the average, one per cent of the volume in the tank. The working volume of a tank for either batch or continuous cultivation is approximately half the total volume.

Operation

Batches of up to 20 litres of medium can be sterilised in average-sized autoclaves and the containers handled easily. Sterilisation is a critical step and in our experience most contaminations arise from failure at this point. Stainless steel containers are durable and do not affect the yield of bacteria. Sterilisation is carried out with free steam for 15 minutes followed by 45-60 minutes at 15 p.s.i. The rubber connections are sterilised along with the vessels, being attached to nipples as much as is practicable and with free ends encased in test-tubes.

After sterilisation, the reservoir containers are coupled to the culture vessel and this in turn is coupled to an empty tank to collect the out-flowing culture. In this line a branch is made to a sample collector which can be opened or closed with a simple clip or artery forceps.

The tank is seeded by inoculation through the diaphragm and left for two hours. The motor is coupled to the shaft by a piece of rubber hose or similar fitting. After two hours the motor is started. Air is blown through the tank at a rate of about one fifth of the culture volume per minute. About 10 p.s.i. are needed usually to overcome the resistance in the air filter (an earthenware candle in a metal cylinder). Silent compressors are now available—the type used for driving high-speed dental drills is suitable.

When the culture in the reaction vessel has reached its maximum density, usually in 15 hours, the flowing-through operation begins. Cultures of *P. multocida* can maintain at maximum density a throughput of a quarter of the culture volume per hour. The apparatus can be calibrated before use and the input of medium regulated with nothing more elaborate than simple glass transfusion drip counters.

Static cultures held in the reaction vessel under aeration for 48 hours usually undergo spontaneous lysis.

Media

Several have been tried and there is scope for improvement. Media based on hydrolysates of casein give the best results and these are easier to manipulate in bulk than meat media. The procedures involved are as follows:—

(*a*) Casein Hydrolysate: To 200 gm. commercial casein in a conical beaker, add a mixture of 170 ml. conc. (10 N) HCl and 110 ml. distilled water. Stir quickly to wet the casein evenly before it swells and hardens.

Autoclave at 120° C. for 45 minutes. Cool, and add 5 per cent NaOH solution to bring the pH to 7.0. Add this slowly and do not allow the temperature of the mixture to rise above 30° C.

This gives approximately 2,000 ml. of hydrolysate at pH 7.0.

Add 3 per cent activated decolourising charcoal and reflux at 100° C. for 20 minutes. Remove charcoal by filtration. The resulting straw-coloured solution has a nitrogen content of approximately 10 gm./litre.

(*b*) Yeast Extract: To 450 gm. dried, inactivated yeast add 5,000 ml. distilled water. Bring to the boil. Simmer for five minutes with constant stirring. Cool, centrifuge at 2,000 r.p.m. for 10 minutes in 500 ml. bottles. The supernatant is the yeast extract. The yield should be about 3,000 ml.

(*c*) Pancreatic Digest: This can be made according to Gladstone's method, but "Oxoid Tryptone" is a simple ready-made substitute.

The Medium

Casein hydrolysate	170	ml.
Yeast extract	100	ml.
Tryptone (OXO)	5.0	gm.
Sucrose	2.0	gm.
$MgSO_4\ 7H_2O$	1.0	gm.
KH_2PO_4	2.72	gm.

Na_2HPO_4. $12H_2O$ ... ... 10.745 gm.
Distilled water ... ... to 1,000 ml.
Adjust pH to 7.6.
Autoclave at 120° C. for 12 minutes. Filter.
Adjust pH to 7.2. Sterilise finally by autoclaving.

The final medium contains approximately 270 mgm. N2/100 ml.

Strains

Three strains were grown repeatedly under the conditions described herein; the "Insein" Type I from Burma; a horse-passaged Type I from U.S.A., and an unusual Type I from Australia.

Results

In comparative tests between jars using the sparger and vortex system of aeration, the yield obtained showed no significant difference for any of the media used. Therefore, for mechanical simplicity, the vortex system has been adopted for routine use. To assess yields of bacteria, cultures are grown for 24 hours. The cells are recovered in a Sharples centrifuge and dried in three changes of cold acetone followed by 12 hours in the vacuum desiccator. The yield is expressed as grams of dry bacteria per litre of medium. For rough estimation during growth, each successive tube in the Brown's Opacity Tubes (Burroughs Wellcome) equals 0.1 gm. dry weight of pasteurella per litre, e.g. a density corresponding to tube 7 would yield 0.7 gm. dry bacteria per litre. The Table shows the average yields obtained in a vortex aerator compared with those from conventional flask cultures.

TABLE I
Yields of *P. multocida* Type I in Aerated Media at 37°C.

Medium	Yield in Still Culture gm./litre	Yield in Aerated Culture gm./litre
Ward and Rudd meat plus yeast (1)	0.15	0.6
Partial Casein Hydrolysate (2)	0.2	0.7
Casein Hydrolysate plus Yeast Extract	0.3	0.9
Casein Hydrolysate plus Yeast Extract plus Tryptone (OXO)	0.5	1.9
Casein Hydrolysate plus Yeast Extract plus Tryptone (OXO) plus Silicone	—	0.8

(1) Bain and Jones (1955).
(2) Higuchi and Carlin (1957).

All the media are sufficiently well buffered to prevent much change in pH during growth. The lowest recorded was pH 6.9.

Some additions to the medium have been tried, either initially or after growth has attained its peak about 15-18 hours after seeding. Iron and manganous salts, fermentable carbohydrates, peptones, sulphites, riboflavin, tryptophane, nicotinamide and a peptic digest of blood have all failed to boost growth by very much. No attempt has been made so far to use labile materials such as fresh blood, serum or lysed blood cells. The aim of this investigation has been to produce simply and regularly a dense culture for the manufacture of oil-adjuvant vaccine. The risks of contamination are so high in tropical laboratories that steps to minimise this factor have a dominant place in any plans for production. In a single trial *P. multocida* grew to the usual density in the presence of 1:500,000 crystal violet and 1:100,000 " Actidione ".

Foaming occurs in the vortex aerator but it is not a mechanical problem. It does not appear to interfere greatly with gas exchange because the yield is the same as in a fully aerated sparger aerator. Silicone antifoam emulsion has been tried, but the cell harvests have been depressed instead of increased.

The protective power for mice of the bacteria grown in sparger or vortex aerators does not differ from that of agar-grown bacteria, i.e. 50 micrograms of either kind of bacteria protects about 80 per cent of mice against 100 L.D. challenge 21 days after inoculation.

Dissociation of the bacteria during growth has not been observed in any of those strains which are used regularly. Capsules remain visible. Chemical studies to be reported on later show that, as would be expected, there is leaching of the surface antigen particularly the polysaccharides into the medium.

The formalised dense cultures emulsify readily with mineral oil. As reported by Bain (1957), the addition of a small amount of Arlacel " A " Special (mannite mono-oleate) to the oil and lanoline leads to greater stability.

Discussion

Not much has been done on the nutritional requirements of *P. multocida* since the work of Berkman (1942), who showed that pantothenate and nicotinamide or diphosphopyridine nucleotide were essential for growth of a wide range of pasteurella in a basal medium of hydrolysed gelatin. An unknown factor present in a concentrate of biotin was needed additionally by a few strains and a further undefined substance (or ratio of substances) in the gelatin hydrolysate. Blood and serum enhances the growth of pasteurella in ordinary cultures. Sterne (1956) uses laked horse blood in an aerated system for continuous cultivation of *P. multocida* and gets havests comparable to our best.

The yield of bacteria seems to depend on the medium rather than on the properties of the aerators. During our experiments in the same apparatus we have only made substantial increases in yield by alteration of the basal medium. In the early trials with meat medium the best yields were constant at about 0.6 gm./litre; casein hydrolysate (acid) as the basal medium raised the level to around 1.0 gm./litre and the addition of pancreatic digest of casein has brought it to nearly 2.0 gm./litre. Yeast extract and salts were the same in all media. Our routine results do not suggest that the highest yields are directly related to the nitrogen content or to

tryptophane. It is interesting that the three strains in regular use behaved similarly in the various media.

A notable feature of the best medium is that under ordinary still culture conditions a density can be attained which is almost the lowest acceptable for blending directly into oil emulsion. Even for the manufacture of plain bacterin, this would be a big improvement on the usual media, provided that there was no toxic effect.

In this laboratory, the glass model with a five-litre reaction volume has produced regularly over a litre an hour of high-density culture for 24-hour periods. The larger steel tank with a 12-litre reaction volume has produced three litres an hour for periods up to five hours, the longest tested.

Occasionally, for reasons unknown to us at present, a failure occurs and a batch attains only a third of its expected density.

An improved model of the steel tank is now being made commercially in Australia.

Acknowledgements

We wish to thank Messrs. A. Audet and H. Sinclair, of the Department of Veterinary Physiology, for their help in building the equipment.

Summary

1. A stainless steel vortex aeration vessel is described for the continuous or batch cultivation of *Pasteurella multocida* Type I.

2. In a casein hydrolysate medium, yields of up to 2 gm. dry weight of bacteria per litre have been obtained.

3. The formalised culture is suitable for the direct manufacture of oil-adjuvant vaccine.

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PROBLEMS OF SLAUGHTER AND MEAT INSPECTION AND THE NEED FOR THEIR INVESTIGATION BY THE RESEARCH WORKER

PART II

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3. Colour Changes in the Liver of Slaughtered Animals

On occasions we have encountered batches of cattle and pigs slaughtered on the same day, many of which exhibited light, sometimes almost clay-coloured livers,

but in none of the animals was there any inflammatory change in the udder, uterus, lungs, intestines or tendon sheaths which might have accounted for this liver change. When such occurrences were observed they were attributed, admittedly somewhat empirically, to the fact that these animals may not have been supplied with food and water during their journey to the abattoir or during the detention period in a lairage, and the term " hunger liver " was therefore coined for this condition. The functions of the liver in the living animal and the possible ætiology of this so-called " hunger liver " are discussed below.

The liver is the largest organ in the body and possesses the greatest number and variety of functions, and the highly specialised hepatic epithelial cells are normally in contact with about 25 per cent of the blood of the body. One of the functions of the hepatic cells is the formation and storage of glycogen, and when glycogen becomes depleted from other tissues of the body, it is the liver glycogen which mainly makes up this deficiency.

Generally speaking, the change in the colour of a liver from a reddish-brown to a lighter or clay-like colour is due to the excessive accumulation of, or appearance of, visible fat in the organ; this may occur physiologically as in animals which are being fattened, in pregnant animals, in castrated males and in brooding hens. An excessive infiltration of fat into the liver may occur also under pathological conditions, as in the over-feeding of animals or when disturbances of nutrition cause a decreased oxidation of fat.

A typical example of disturbance of nutrition giving rise to a fatty infiltration of the liver is the pregnancy toxæmia of ewes, for the sheep apparently has a low carbohydrate reserve and the extra demand made on the ewe in late twin or triplet pregnancy depletes the glycogen reserve and fat replaces it. In such cases there is a striking change in the liver colour, which is understandable when it is realised that the amount of fat in the liver may be increased from the normal three to four per cent of the fresh weight of the liver to as much as thirty-five per cent in animals which are affected with this disease.

Fatty infiltration is a reversible change and if the condition which causes it should subside the cells recover and the liver colour returns to normal. A more serious condition is the so-called fatty degeneration which may arise as a result of septicæmic or toxæmic diseases, for here the accumulation of fat in the liver cells appears to result from actual damage to the cells. Cells thus affected may die and the change in fatty degeneration is then an irreversible one. It will be obvious that a change in liver colour to a lighter hue can be a useful guide to the inspector as indicating that a septicæmic or toxæmic affection may have existed at the time of slaughter.

When we first encountered light coloured livers in slaughtered animals the carcases of which exhibited no other change we were led to believe that this might be attributed to starvation together with lack of water (for the injurious effects of starvation are increased if water is withheld), because the starving animal attempts to maintain its body heat and functions by drawing upon the necessary constituents of its own body; the most easily oxidised materials of the body are glycogen and fat and it is known that these are used up first and that these held in the liver are

withdrawn. It is, however, questionable whether starvation alone could account for a change in the liver colour to a lighter hue. Robertson and Thin (1953) have shown that a few days complete starvation in steers, dry cows when non-pregnant, or in cows producing only a little milk seems to have little effect on fat metabolism as judged by ketone-body production. There is, of course, a considerable loss in *liver weight* during starvation, and Callow (1956) has shown that in sheep lairaged for two, three or four days this loss is considerable and progressive, even when water and ample hay is available to them. In this case the loss in liver weight is probably explained by the fact that the sheep starve themselves voluntarily because of their inability to settle in their strange surroundings.

These experiments are mentioned because in neither of them is there any record that the starvation resulted in any significant colour change in the liver, and our own observations eventually led to us to the conclusion that some other factor might be responsible for this colour change. A clue was provided when a number of sheep which had been worried by dogs and severely injured were sent immediately to a slaughterhouse in Newcastle upon Tyne, and of the sixty sheep slaughtered practically all of them showed a colour change in the liver to a yellow or yellowish-brown. The dogs had apparently been busy throughout the night and the sheep subjected to very considerable exertion, stress and physical pain. It therefore appeared that " hunger liver " might be caused by other factors, or a combination of starvation and some other factor, and one of these might be physical pain. During rough treatment (Plate 3) or long wearying transport, animals are subjected to considerable stress and pain, and under these conditions there would be depletion of the muscle glycogen and as a result glycogen would be withdrawn from the liver to maintain the blood sugar level.

It would not be expected that the physical stress and degree of glycogen depletion would be the same in every animal undertaking a particular journey and it is perhaps significant that in cattle consigned to an abattoir in Ireland it has been noticed that a yellow change in liver colour is rarely observed in animals which have come from districts where the grazing is poor. The explanation of this might well be that such animals, having roamed considerable distances in their search for food, are in better " training " than are housed animals and as a result their muscles will be less readily depleted of glycogen during long journeys. It is a fact known to every livestock trader that grazing animals such as horses, sheep and grass-fed cattle are affected less deleteriously by transport than are stall-fed cattle, pigs and suckling lambs.

The work of Engdahl (1950) at the abattoir in Helsinki is illuminating when considered with the aforementioned observations. He lays particular emphasis upon the susceptibility and response of the liver, not only in acute infectious diseases and intoxications but also in deficiency diseases and in distressing abnormal conditions such as fractures, contusions, long wearying transport, debility and starvation, and suggests that these may be responsible for changes in liver colour. In cases, therefore, where the liver shows a lighter colour but is not enlarged and there are no other abnormalities in the carcase he suggests that the presence of a septicæmic condition may be discounted and attention directed towards one of the aforemen-

tioned causes. If the liver is swollen, however, or is pale in colour with a reddish tint, septicæmia should be suspected, especially if the spleen is also enlarged.

In order to demonstrate and measure changes in liver colour, Engdahl (1951, 1953) used an apparatus on the photo-cell principle and found it of value because in Finland fifty per cent of all the animals killed are slaughtered in rural districts and the carcases may then be consigned to the consuming population in the towns. In such cases the veterinary inspector in the town has no opportunity to carry out an ante-mortem inspection but the law requires that the carcase must be accompanied by the head, tongue, heart, lungs, spleen, liver, udder, uterus and kidneys. Under such conditions of inspection liver changes are of undoubted value to the inspector, but Engdahl remarks that though a light coloured liver is an indication of abnormality, the colour change may be but a slight one in sub-acute and chronic diseases.

With the aid of the photo-cell apparatus Engdahl has measured the liver colour in various abnormal conditions and has come to the following conclusions:—

A. *Cattle*

A colour index of 2 corresponds to the colour of dark chocolate, an index of 8 to colour of light mahogany and an index of 30 to a pale yellow or clay-like colour. The colour index of the normal liver in cattle is 3-6, while that of the suckling calf may have an index as high as 30. In healthy cows in the last months of pregnancy the colour index may reach the figure of 7-8. *Dark coloured livers* have an index of 2-3 and are (*a*) smaller and firmer in old senile cows, (*b*) of normal size in xanthosis, and (*c*) enlarged, particularly the caudate lobe which is also rounded, in cattle slaughtered on account of tympanitis following on a feed of clover (he records 88 cases). *Light coloured livers*, usually not enlarged, (*a*) have an index 7-9 and are seen in cattle slaughtered after exertion, hunger (14 cases), contusions, fractures (23 cases), in the earliest stages of acute infectious diseases and in chronic infectious diseases (110 cases), (*b*) have an index of 8-15 and are seen in cattle slaughtered on account of milk fever (22 cases), prolapsus uteri (8 cases) and coli mastitis (4 cases), and (*c*) have an index of 10-20 in cattle slaughtered on account of puerperal hæmoglobinuria (14 cases) and piroplasmosis (10 cases).

B. *Horses*

The colour index of the normal liver of horses is 2-3. In horses up to three years of age the colour index may be as high as 10. (*a*) A colour index of 5-8 is seen in colic (22 cases), severe contusions and old fractures (35 cases), and (*b*) a colour index of 5-20, with the liver usually enlarged, is seen in azoturia (20 cases).

C. *Pigs*

The colour index of the normal liver in fattening pigs is 6-8; in unfattened sows and breeding pigs it is 2-6. The liver has a colour index of 8-20 and is therefore light coloured, though not swollen, in cases of severe fractures necessitating slaughter, severe contusions (77 cases) and prolonged and wearying transport. The liver is normally dark coloured in pigs which are in store condition, is dark red in badly bled animals (and in such cases blood drips from the organ), red and swollen in infectious diseases, gray and sour smelling in decomposition, gray but odourless if water-soaked, and hard and swollen in chronic diseases.

The work of Engdahl confirms our contention that animals subjected to severe untoward conditions shortly before slaughter may show significant changes in liver colour and that the term "hunger liver" is only partially a correct one. Our conclusions are as follows, that (*a*) the liver of slaughtered animals is liable to changes in colour that cannot be attributed to infection, fever, parasites, toxic substances or to post-mortem changes, (*b*) untoward conditions such as severe stress, fractures or strenuous transport may cause depletion of liver glycogen and be responsible for this colour change, (*c*) the feeding of the animal during the period prior to a journey may be of importance and though the term "hunger liver" may be only partially a correct one it is possible that starvation is a contributory ætiological factor, and (*d*) a liver with a colour lighter than normal is an indication for a particularly thorough examination of the carcase, as quite apart from the fact that this may be a manifestation of a systemic change, it indicates that there may have been a drain on the muscle glycogen resulting in a high pH of the flesh and a lowering in the durability of the carcase. Engdahl remarks significantly that in 116 slaughtered cattle in which no change other than a light coloured liver was observable, in 54 of these cattle the pH of the flesh was 6.4 or more.

4. The Significance of Small Hæmorrhages in the Kidneys of Slaughtered Pigs

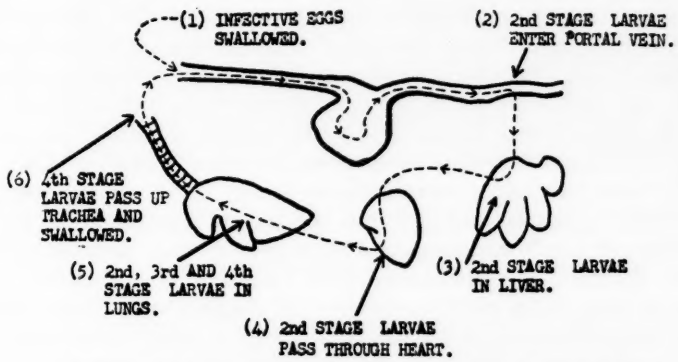
Capillary hæmorrhages are due to a variety of causes, among these being septicæmia, toxæmia and certain vitamin deficiences. In septicæmia the bacteria may lodge in the capillary and cause direct injury to its endothelium. The products of some pathogenic bacteria and viruses will also damage the endothelial wall and a typical example of this, known to every student and meat inspector, is the typical "turkey egg" kidney of pigs affected with acute swine fever, with petechial hæmorrhages situated beneath the kidney capsule. The venoms of some snakes and other chemical substances are toxic and in sufficient concentration will damage the capillary walls.

In all animals the presence of capillary hæmorrhages in the kidney and elsewhere is a most suspicious sign when detected by the meat inspector and draws his attention to the possibility that the hæmorrhages are a manifestation of a septicæmic or toxæmic condition. The inspector, therefore, looks immediately for the primary focus as a cause of the petechial hæmorrhages and in this he may be successful. Experience shows that such hæmorrhages in pigs are frequently caused by an acute arthritis, particularly of the hock, stifle or knee, and there are numerous other systemic infections which will give rise to petechiation of the kidney cortex.

In these cases where there is an obvious association between a primary infective focus and the presence of petechial hæmorrhages the task of the inspector is a simple one, for he will rightly regard the carcase as unfit for food. Not infrequently, however, a few small hæmorrhages are found in the kidney of a slaughtered pig and careful examination of the carcase of offal reveals nothing which could be regarded as a causative factor—in other words, a few kidney hæmorrhages are found in a normal carcase without any evidence of lymph node enlargement, fatty change of the organs, or imperfect bleeding, and in routine meat inspection such cases cause a deal of worry to the inspector in whom is vested the responsibility

of condemning or passing the carcase. These isolated hæmorrhages in the kidneys of normal pigs have been attributed by some authorities to an irritant of dietetic origin, but this is a far from satisfactory explanation and the purpose of this contribution is to draw attention to two possible causes, namely (*a*) the action of the larval forms of *Ascaris lumbricoides var. suillæ*, or (*b*) that the kidney hæmorrhages are of mechanical origin and caused by capillary rupture in pigs slaughtered by electrical means.

FIG. 4


(1) INFECTIVE EGGS SWALLOWED.
(2) 2nd STAGE LARVAE ENTER PORTAL VEIN.
(6) 4th STAGE LARVAE PASS UP TRACHEA AND SWALLOWED.
(5) 2nd, 3rd AND 4th STAGE LARVAE IN LUNGS.
(3) 2nd STAGE LARVAE IN LIVER.
(4) 2nd STAGE LARVAE PASS THROUGH HEART.

Life history of Ascaris lumbricoides, the common roundworm of pigs.

a. *The action of the larvæ of Ascaris lumbricoides*

The life history of this common roundworm of the pig is well known (Fig. 4). After the larvæ pass through the intestinal wall of their host they enter the branches of the portal vein and are transported to the liver. Here some larvæ may be arrested and by their irritation give rise to hæmorrhages, particularly around the intralobular veins, and necrosis of one or more liver lobules; this is followed by absorption and repair with the deposition of fibrous tissue and the production of whitish foci with an indefinite boundary (the so-called "milk spots") on the surface and in the substance of the organ. It is probable that most of the larvæ emigrating from the intestine pass through the liver and are carried by the blood of the posterior vena cava to the lungs, where in order to complete their life history the larvæ must break through a lung capillary into a smaller bronchus and be wafted up the trachea by coughing and the aid of the ciliated lining epithelium. All the larvæ, however, do not complete their life history in this way, for some larvæ which have reached the lungs and are assembled in the capillary blood vessels may be swept onwards into the general circulation and be carried by the arterial blood stream to other organs of the body, such as the heart or kidneys. Here these larvæ, just as in the liver, may cause hæmorrhage, necrosis and fibrosis so that small hæmorrhages in the kidneys of pigs may be due to the destructive action of fourth-stage Ascaris larvæ on the capillary walls of that organ. It must also be remembered that Ascaris larvæ can irritate tissues by the toxic substances they secrete, and the observations of Mönnig (1947) are significant when he states that "Ascaris

larvæ which find their way into the general circulation may cause small hæmorrhages in important organs like the brain, myocardium and kidneys, but some of the lesions caused in these organs are probably due to toxins. Toxic lesions in the kidneys may lead to necrosis of the tubular epithelium and the presence of casts in the urine ".

b. *Hæmorrhages in the Kidneys of Pigs caused by Electrical Slaughter*

Every year greater use is being made of the electrical apparatus for the stunning of pigs prior to their bleeding. In Britain it has been found to be uniformly satisfactory and successful, though it is true that unless there is careful control of (*a*) the point of application of the apparatus to the head, (*b*) the voltage, and (*c*) the time of application of the current, the method may be anything but a humane one. It is nevertheless surprising to find that in certain countries objections have been raised to this method of slaughter, chiefly on the grounds that it sometimes caused hæmorrhages in certain of the body tissues. In 1954, legislation was introduced in Denmark enforcing electrical stunning of pigs, but in March 1955 the Danish Ministry of Justice issued a circular stating that bacon factories which made application could be exempted from the law enforcing electrical stunning. This " change of face " was occasioned by a petition received from the meat packers in Denmark who complained that " stunning with electricity causes extravasation in the meat, bloody intestines, and fractures in the spinal column, pelvis and shoulder blades through shock. The blood in the meat makes it more susceptible to putrefaction and has a detrimental effect upon its taste ". It may be pointed out that the term " extravasation in the meat " is synonymous with the term " splashing " used in this country and denotes the presence of hæmorrhages in certain tissues and organs of the slaughtered animal where that animal has been mechanically stunned; in pigs bled while suspended it is the lungs that most frequently show this slight trace of splashing in the form of pin-point hæmorrhages, particularly along the dorsal border of both lungs. These hæmorrhages, which are bright red in colour, are best seen beneath the visceral pleura but they also occur in the substance of an involved lung and are caused by an escape of blood into small groups of alveoli (Plate 4); occasionally the hæmorrhages are present in such numbers that they give the impression of a single large lesion. Splashing may also occur in the muscular tissue, in the epicardium, which, like the lungs, is particularly sensitive to shock, and it may also occur in the intestines and occasionally in other organs.

Investigations made in Denmark, presumably as a result of the complaints made by the meat packers, have shown that splashing of the musculature could be obviated for all practical purposes if bleeding were commenced in not more than five seconds after the termination of stunning, whereas if the interval between stunning and bleeding was extended to 30-45 seconds it was found that splashing of the musculature occurred in 0.5 to about 5 per cent of slaughtered animals (Jul, 1957).

The reason that hæmorrhages occur in the tissues of animals as a result of slaughter must now be considered and it is important to realise that the endothelium lining the capillaries is very sensitive to lack of oxygen so that any condition that reduces the supply of oxygen to the body for even a short time will, before death

PLATE II

FIG. 3

Weighing a live pig by hanging it on a primitive form of steelyard at a slaughterhouse in Burma. Rough treatment of this nature prior to slaughter would be likely to cause rapid depletion of muscle glycogen and lead to deleterious changes in the muscular tissue.

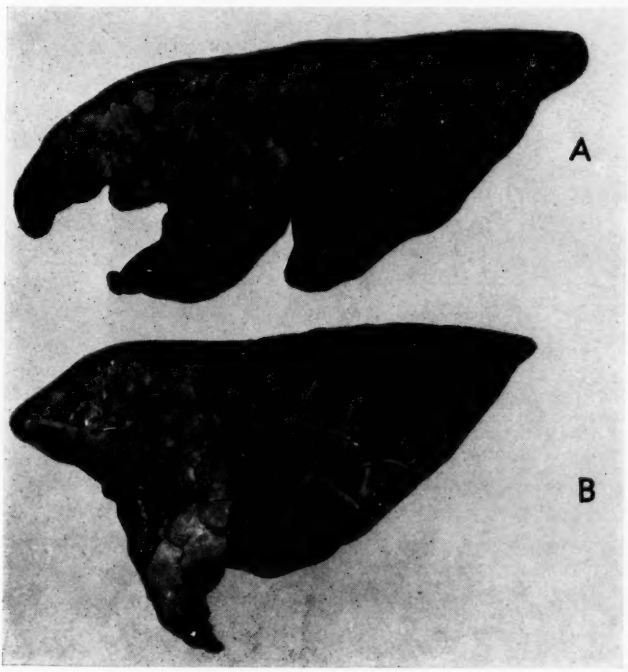


FIG. 4

A.—Lung of pig showing numerous hæmorrhages which occur in animals stunned by electrical means, particularly if the pig is hoisted prior to bleeding. B.—Lung of pig also stunned electrically but in which no hæmorrhages have occurred.

(*Article by H. Thornton, on page 220*)

PLATE III

FIG. 5

Carcase of pork showing well marked transit erythema caused by exposure of the live animal to the cold air during transport by motor vehicle to the slaughterhouse.

FIG. 6

Cow being bled after stunning with captive-bolt pistol. The stunned animal is unable to retract its head and the flow of blood from the sticking wound is therefore profuse and uninterrupted.

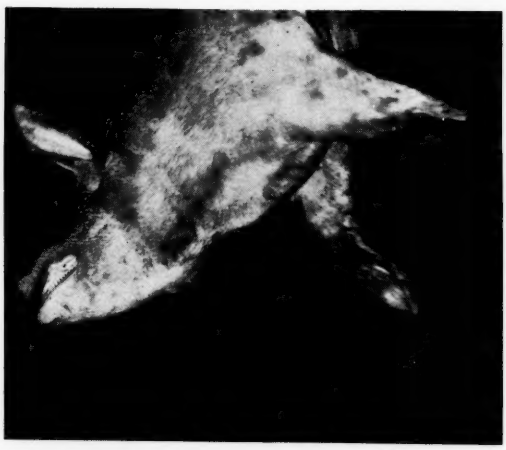


FIG. 7

Pig being bled suspended but without previous stunning. Note the marked retraction of the head, which may hinder uninterrupted egress of blood from the sticking wound and facilitate hæmatogenous distribution of contaminating organisms.

(Article by H. Thornton, on page 220)

occurs, injure the capillary walls and render them liable to rupture. In addition, if generalised spasm of the skeletal muscles occur there is a marked increase in venous pressure with a consequent increase in pressure within the capillaries and a stretching and thinning of their walls. The hæmorrhages which occur in the lungs and other tissues of electrically stunned pigs are the result of (*a*) the anoxia, or oxygen lack, which follows on the stoppage of respiration and which is itself induced by contraction of the muscles of respiration (spasm of the glottis may also be a factor in causing cessation of respiration), and (*b*) the increase in capillary blood pressure due to the tonic, tetanus-like convulsions which occur in electrical stunning. (In pigs anæsthetised by the carbon dioxide method no hæmorrhages occur in the lungs or muscular tissue.)

One might refer at this juncture to the avowed objection made in the United States to the adoption of the stunning of pigs by electrical means. The use of electrical stunning methods in plants which operate under Federal meat inspection "has not been permitted as a result of experiments which were conducted at the University of Chicago. These experiments indicated that electrical stunning of hogs resulted in certain changes in the tissues which could not be differentiated by gross examination from similar changes produced by a diseased condition". The diseased condition referred to was hog cholera, the American term for swine fever, and the changes which were produced by electrical stunning were the hæmorrhages in the lung tissues already described.

Though the lungs are the main seat of hæmorrhages found in pigs stunned electrically and subsequently bled while suspended, the report also states that "in a very small number of animals that had been electrically stunned petechial hæmorrhages were found in the pericardial covering of the heart surface, but the number of animals in which this lesion was present was so small as to be negligible". The report goes on to say "in a small percentage of electrically stunned hogs an occasional petechial hæmorrhage was found immediately beneath the capsule of the kidneys and the number of animals showing this lesion was greater than the number showing hæmorrhages in the epicardium".

It is suggested that the occurrence of subcapsular petechiæ in the kidneys of electrically stunned pigs may be related in some degree to the anatomical structure of this organ, for the kidneys are not protected against the effect of increased venous pressure caused by the muscular contractions induced at the time of slaughter. The renal arteries are short and are set at right angles to the aorta from which they arise, and the pressure in the capillaries of the kidney glomeruli is known to be higher than the general systemic circulation. Similarly, the renal veins are short and enter the posterior vena cava almost at right angles and there is evidence that in electrical stunning the venous pressure rises more rapidly and to a greater height than that seen in stunning with the captive-bolt pistol (Clark and Tweed, 1932). Thus increased pressure in the vena cava due to circulatory disturbance would be be transmitted through the renal veins to the kidney capillaries and might cause rupture of the capillary walls.

It does not seem likely that fourth-stage Ascaris larvæ will produce very numerous lesions in the kidneys of pigs harbouring the adult worm in their intestines

and there is no evidence that electrical stunning produces other than an occasional odd hæmorrhage in the kidneys of pigs which are slaughtered by this means. When petechial hæmorrhages are very numerous in the kidneys of pigs they may almost invariably be attributed to the presence of an infective agent in the blood stream, but the inspector's work may be facilitated if he recollects that odd hæmorrhages which occur in the kidneys of otherwise normal pigs may possibly be due to Ascaris larvæ, and in this case the destructive action of the larvæ may also be apparent in the liver and pulmonary tissue. On the other hand, the hæmorrhages may be of mechanical origin and caused by the shock induced during slaughter and in such cases may be accompanied by petechiation of the pericardium covering the heart surface.

5. Congestion of the Bronchial Lymph Nodes of Pigs Due to Mechanical Causes

The bronchial lymph nodes of the pigs vary in size and are up to 1 cm. or more in diameter. Normally they are pale pinkish-grey to a pale red in colour and somewhat soft in consistency.

Any change of an acute infective nature in the lungs of pigs will, of course, be manifested by an enlargement and congestion of the bronchial nodes. This can be seen daily in any bacon factory, for there is invariably quite a high percentage of animals found to be affected with lobar pneumonia, and it should be noted that in such cases the nodes are not only congested but are also enlarged, the congestion being a manifestation of an active hyperæmia, while the enlargement is due to œdematous changes. It is, however, common in routine meat inspection to find that the bronchial nodes of pigs are congested though not enlarged and without any evidence of any acute infective process in the lungs which would account for this congestion. This occurs particularly in animals which have been electrically stunned and which show hæmorrhages in the lung tissues already described, and in some cases there is a tendency for the congestion to be limited to the cortex of the node, thus producing a red ring about the periphery.

From the very frequent association in pigs of lung splashing and engorgement of the bronchial lymph nodes there would appear to be little doubt that blood extravasated as a result of capillary rupture enters the lymph stream and is rapidly drained into the node which drains the area. A point of considerable interest to the meat inspector is how soon a lymph node can become suffused with blood by its drainage of an area where hæmorrhage has occurred. This drainage would occur very rapidly in organs such as the lungs and kidneys, and when the bronchial nodes are affected in slaughtered pigs this must occur during the time that elapses between stunning and bleeding. In the electrical stunning of pigs, when the tongs are in position for only three to ten seconds, and bleeding, at any rate in bacon factories, is commenced in 45 seconds, the presence of lung splashing is often accompanied by a congestion of the bronchial nodes.

It has also been our experience in the days before mechanical stunning of pigs became obligatory to encounter occasional cases where a pig has been hoisted from the ground for the purpose of the neck cut but has fallen from the hoist and in doing so has fractured one or two bones. In such cases the animal was rehoisted

immediately and was bled within a space of one to two minutes at the most, but invariably the nodes draining the injured area appeared suffused with blood and thus further evidence is provided that hæmorrhages which occur in pigs no more than two minutes before the animals are bled may manifest themselves in the form of blood engorgement of the associated lymph nodes.

It is obvious that meat inspectors should be aware that hæmorrhagic foci which occur just prior to, or as a result of, slaughter may be drained very rapidly by the afferent lymphatics of the area in which the hæmorrhage occurs and that this will result in blood infiltration of the associated lymph node. In meat inspection it is therefore of the greatest importance to differentiate between the normal-sized node which has become infiltrated with blood from a nearby hæmorrhagic focus, and the node which is congested and enlarged due to some infective or toxic agent.

6. Skin Discoloration in Pigs Due to Mechanical Causes

Every inspector is acquainted with the condition which occurs in pigs when local areas of skin are congested, often in pairs of lines parallel with each other, and which is due to an injury caused by the hooves of one pig which climbs over another, sometimes during detention in a lairage, but more frequently during road transport. Likewise, every inspector recognises the more extensive skin discoloration or rashes caused by febrile affection such as acute swine erysipelas, pig paratyphoid or swine fever, a feature of such cases being that the skin lesions become more obvious after the pig has emerged from the scalding tank.

There are, however, other causes of skin congestion in the pig, some of which are due to intrinsic factors and others due to mechanical influences, but at times the inspector is puzzled as to the apparent cause. In pigs an urticaria is sometimes seen and is believed to be due to an excess of protein or to some dietetic errors or deficiency. The spots are initially the size of a lentil or larger on the lateral and under parts of the chest, but sometimes the whole body is affected, and in a few days the red patches have extended to the size of a small saucer and are raised, whitish in the centre, with a red border. The swellings may be rounded but sometimes they are quadrilateral and then resemble an erysipelas urticaria. We have noticed that the affection may occur when fattening pigs are first put in to potato feed, while in the United States it is recorded that certain feeds, such as buckwheat, may cause urticaria in older pigs and that the affection is a reaction of an allergic nature.

It is probable that exposure to a cold draught during road transport will cause skin congestion, and transit erythema (Plate 5), as this is called, is not uncommon; on the other hand, excessive exposure to the sun can cause erythema, but this, though it occurs in other animals, is only observed readily in the pig with its white unpigmented skin. Because of this susceptibility of the white-skinned pig to solar erythema, black-skinned pigs such as the Large Black or Berkshire are sometimes preferred when the animals are to be pastured or field-fed. The condition known as lime burn or disinfectant burn was at one time fairly common in pigs travelling long distances in railway trucks, the congestion appearing as red patches along the belly and ham where the animal had been in contact with some irritant substance on the floor; the affection now rarely, if ever, occurs.

The undue activity of a pig prior to slaughter may also be a cause of skin congestion, as in the case of a " distressed " pig which has been chased prior to slaughter or has walked some distance uphill to the abattoir. The pig arrives panting and exhausted and if it is of a white breed the skin shows a uniform pink discoloration after the carcase emerges from the scalding tank. In such cases there will be a varying degree of congestion of the lymph nodes and this confirms our previous observation of the rapidity with which such congestion can occur. If therefore one encounters a number of " splashed " carcases with congested lymph nodes one should suspect a lack of rest prior to slaughter or some violent excitement immediately prior to stunning; practical experience shows that if the pig is given a short rest or a spray of cold water prior to slaughter these untoward changes will not occur.

Cases sometimes occur when pigs, even though mechanically stunned, are immersed in the scalding tank before all movement has ceased, and on emerging the animal shows a uniform pink coloration of the skin. On the occasions we have encountered this the slaughterman usually proffers the information that the pig " kicked when placed in the scalding tank " indicating that the animal must have been ineffectually stunned and that the heart was still functioning. One can also recollect cases where due to inadvertence a pig has jumped into scalding tank and when retrived, stunned, bled and scalded has shown a uniform congestion of the skin.

In order to ensure that pigs shall be lifeless before they are immersed in the scalding tank the Meat Inspection Division of the Bureau of Animal Industry of the United States specifies that " hogs shall be bled and shall hang on the bleeding rail for a period of not less than six minutes ", and in Britain when pigs are about to be immersed in the scalding tank it is common to observe that the slaughterman splashes the ear of the suspended pig with some of the scalding water to satisfy himself that all reflex movements have ceased before the animal is immersed. In the United States a reddening of the skin of pigs is frequently observed in animals which have passed through the mechanical dehairing machine; this skin congestion is attributed to the effect of the mechanical beaters and rough treatment generally in the machine subsequent to scalding, the residual blood being found in the capillaries of the subcutaneous tissue.

It will be obvious that skin discolouration occurring in pigs due to mechanical causes may worry the inexperienced inspector, but he should recollect that though in skin discoloration of mechanical origin the lymph nodes may also be congested they will not be enlarged and there will be a complete absence of any pathological or any other abnormal change in the carcase or organs. The flesh cannot be considered objectionable from the point of view of fitness for human food, though the removal of the skin is indicated on æsthetic grounds and because the skin of the pig constitutes part of the joint of pork.

7. Bone Taint

This term denotes the development of a putrefactive process in the deep-seated musculature of the carcase, usually near the bone. The condition occurs in both

cattle and pigs and in these animals the taints are fundamentally similar in origin and are due to development of large numbers of bacteria which probably originate from the intestines of the animal themselves.

The two practices applied in slaughterhouses to diminish the number of bacteria in meat are to eviscerate and to bleed the carcase, for the former probably removes the main source of the bacteria, while the latter reduces their spread throughout the tissue. When bacteria are found throughout the muscle of a freshly slaughtered animal, however, it is still doubtful how many of these bacteria were present in the living animal, how many escaped from the intestine into the blood stream during the agony of death, and how many might be introduced into the blood during the act of bleeding.

It is known that intestinal bacteria can invade the blood stream ante-mortem, and there is considerable evidence that fatigue, fright, shock, slow bleeding or even a sudden strain may facilitate the passage of bacteria through the wall of the gut and into the blood stream and muscular tissue. That fatigue is an important factor in the ætiology of bone taint in both catle and pigs is confirmed by trade experience, and a factor which must also be considered is that walking cattle up a steep ramp to the top floor of a " frigorifico type " abattoir may cause damage to the hip region and be a predisposing cause as also may the shackling of the hind leg of cattle and pigs to raise them to the bleeding rail. In pigs the attachment of the shackle around the hock not infrequently leads to trauma in the region of the acetabulum, though in our experience this trouble may be obviated almost entirely when the shackle is attached to the fetlock region. Indeed, one large firm in the United States has recently adopted a prone-sticking, no-shackling process for the slaughter of their pigs and reports that the method has fully proved itself inasmuch as it has resulted in a 90 per cent reduction in internal ham trauma. It is perhaps significant also that when bone taint occurs in hindquarters of home-killed beef, the condition is stated to commonly involve the side on which the animal has fallen after stunning. The writer (Thornton, 1951) has discussed the effect of traumatism in the ætiology of bone taint and has observed that in some cases of bone taint in beef there has been present rupture or strain of the tendons inserted into the upper processes of the femur, this causing injury to the periosteum and rupture of the small arteries which supply the bone marrow and spongy tissue within the bone. Once a hæmorrhagic focus develops within the bone any anærobic spore-forming bacteria present will multiply rapidly and, if conditions are favourable, will extend centrifugally through the bony tissue by way of the small nutrient foramina and then into the adjacent muscles. It is the opinion of meat traders in New Zealand that bone taint in beef originates in the blood vessels of the bone marrow of the femur.

As previously stated, the bacteria responsible for bone taint may be present in the tissues before the animal is slaughtered and there is also the possibility that the contaminating organisms may be introduced into the carcase at death, possibly by the sticking knife; in the case of pigs there is a further possibility that contaminating bacteria may gain access to the carcase by way of the neck cut when the carcase is immersed in the scalding tank. The work of Lepovetsky and others (1953) on the microbiology of lymph nodes, bone marrow and muscular tissue of slaughtered

cattle showed that bacteria could be isolated from these tissues of animals which had been freshly killed. Experiments were conducted on 23 cattle, and in eleven of these the prescapular lymph node, humerus and muscular tissue bordering the bone were removed from the forequarter, while in twelve cattle the popliteal node, femur and neighbouring musculature were removed from the hindquarters. Bacteria resembling those recognised as important in bone taint were found as follows:— In 15 out of 23 lymph nodes, in three out of 23 marrow samples, and in two out of 23 muscle samples. Thus the number of bacteria found in the lymph nodes was greatly in excess of those found in marrow or muscle and Lepovetsky suggests the possibility that in bone taint the spoilage process may originate from the popliteal or prescapular lymph nodes. (Similar work has been done on lymph nodes removed from human corpses and showed that a large proportion of nodes contained a variety of bacteria, the suggestion being that the bacteria found in the nodes may have gained access to the body through skin abrasions, becoming localised in the nodes and surviving there for some time.)

The relative freedom from bacteria of the muscle tissue and bone marrow of freshly slaughtered cattle is in contrast to the work done in the United States by Jensen and Hess (1941) on slaughtered pigs. These authorities consider that the difference in the location of bacteria in freshly slaughtered cattle as compared with pigs may be related to the different methods of slaughter, for cattle are stunned before being bled and are therefore unable to retract their heads (Plate 6), whereas pigs in the United States are largely bled suspended but without previous stunning and when the neck cut is made and the anterior vena cava severed they retract their heads and thus prevent the egress of blood (Plate 7). Jensen and Hess conclude that when pigs are slaughtered in this way there is ample opportunity for the hæmatogenous distribution of organisms to muscle and bone marrow and that this accounts for the relatively high incidence of bacteria found in the tissues of these animals. The observation may be made here that if retraction of the pig's head is an important factor in preventing egress of blood and also in causing contamination of the tissues of the animal by bacteria introduced into the blood through the sticking wound, it provides further evidence for the advisability of rendering pigs unconscious before the neck cut is made.

The fact that bacteria which have lodged in the popliteal node of cattle may multiply shortly after death of the animal and extend to the surrounding tissue to cause bone taint has a public health significance in relation to the occurrence of human food poisoning. In cattle which have recovered from an acute salmonella infection and have become "carriers" it is common to find that though these organisms are absent from the muscular tissue they persist in the gall bladder, liver, kidneys, spleen and also the carcase lymph nodes and may multiply in the nodes if the carcase is subjected to unfavourable conditions of storage. Indeed, Kafel (1957) has shown that in salmonella-infected pig carcases the organisms, when found in the carcase lymph nodes, could invade the muscular tissue even though the carcase was held for 48 hours at 4° C. If, therefore, a carcase containing latent salmonellæ in the lymph nodes is not cooled rapidly enough immediately after slaughter it is conceivable that the salmonellæ, being facultative anærobes, may multiply con-

cominantly with the anærobic organisms which are the cause of bone taint and may render the adjacent muscular tissue not only æsthetically repugnant, but also dangerous as an article of food. Thus the method by which the meat inspector often deals with hindquarters of beef affected with bone taint, namely by removal of the bones (femur) and generous paring away of the affected muscular tissue, may therefore appear to be a potentially dangerous one, but the evidence is not strong enough to suggest that this accepted procedure should be replaced by that of complete condemnation of the hindquarter irrespective of how far the putrefactive process appears to have extended from the bone or carcase nodes.

Some bacterial contamination must occur at the time of slaughter; whether bacterial growth will occur depends on (*a*) whether the animal was fatigued at the time of slaughter, and (*b*) the rapidity with which the body heat is removed from the carcase. If meat is obtained from an animal which has been rested and there is an immediate lowering of the internal temperature of the carcase to below 20-25° C., this being a temperature range in which the growth of anærobic bacteria becomes seriously retarded, little or no trouble occurs from bone taint even though some bacterial growth may take place down to 10° C. But large fat bullocks are animals which are liable to be fatigued during transport because they are both heavy and fat, and the joints of beef which mainly become affected with bone taint are larger than the other joints of the carcase and are more nearly spherical and the slowest to cool. In hot weather the rate of cooling of such carcases by hanging in normal air may therefore be so slow as to allow bacterial multiplication in the deeper parts.

Refrigeration facilities are by no means universally available in the meat industry and considerable interest must therefore be displayed in the work being done in the use of antibiotics as an aid to prolong the durability of meat foods by delaying bacterial spoilage. The most striking results have been accomplished by the broad-spectrum antibiotics, particularly those of the tetracycline group, but they cannot replace good hygienic methods, which lower the initial bacterial load, while the advantage to be gained by using antibiotics is greatly increased when refrigeration is also used. The use of these substances is extremely simple and adaptable to almost any existing technology, for oxytetracycline (terramycin) and chlortetracycline (aureomycin) are both water-soluble and diffusible and may be injected into animals prior to slaughter or infused into carcases on the killing floor; again, the various cuts of meat may be dipped or sprayed with the solution.

In the presence of an effective concentration of a broad-spectrum antibiotic only a few bacteria present are able to grow and these at reduced rates, and meat treated by infusion with a solution of chlortetracycline may be hung at room temperature with safety for at least 48 hours after slaughter. Spraying the surfaces of carcases as an adjunct to chilling in the long-distance transport of meat to Britain has obvious possibilities as also has the use of antibiotics in lowering the incidence of bone taint in beef hindquarters, but a possible hazard in employing antibiotics for this latter purpose is that an antibiotic completely alters the spoilage pattern so that while retarding the growth of an obnoxious putrefying organism it may allow far more dangerous resistant pathogens to grow unchecked without obvious

spoilage (Barnes, 1957). The application of antibiotics to perishable foods could nevertheless bring about great nutritional improvements in under-developed areas where there is usually a marked protein deficiency in the daily diet and where the lack of refrigeration and prevailing high temperatures have hitherto precluded any possibility of long-distance transport of fresh meat, fish or milk. In the United States in 1955 appeared the first Order permitting the use of an antibiotic for food preservation; chlortetracycline and oxytetracycline may now be used in or on uncooked poultry, and recently the use of chlortetracycline for both poultry and fish was made legal in Canada.

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A MALFORMATION OF THE DUODENAL LOOP OCCURRING IN FOWLS

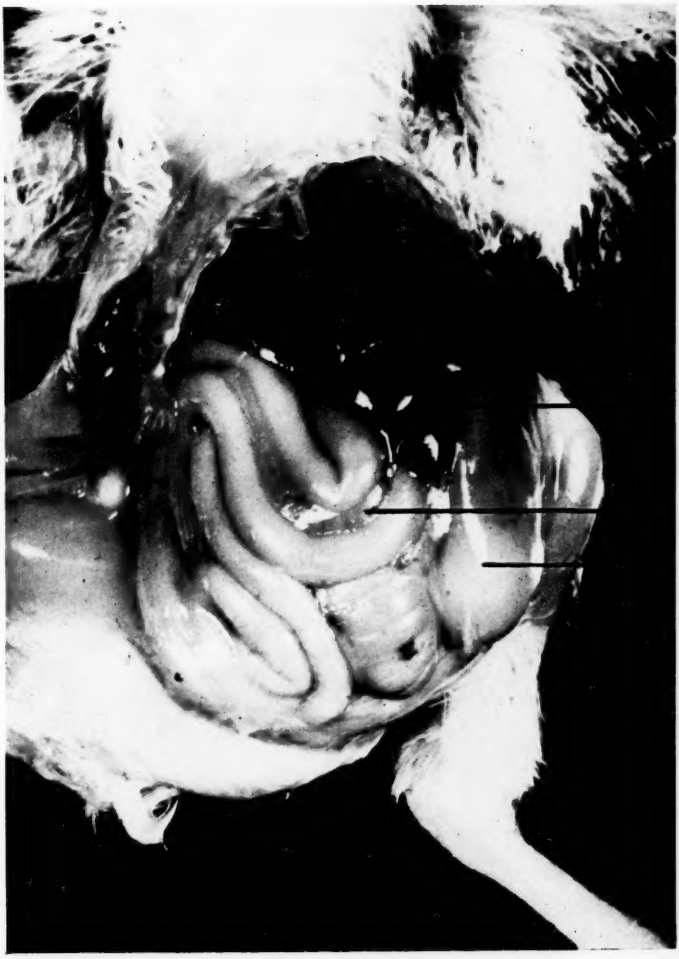
By J. G. CARR

B.E.C.C. Unit, Agricultural Research Council Poultry Research Centre, Edinburgh

In a series of post-mortem examinations of Brown Leghorn fowls of the Centre flock, both normal and experimentally infected with cancer, an abnormality of the duodenal loop was often encountered which does not seem to be mentioned in the literature. Because of its frequent occurrence, and the fact that most of the affected birds were " poor doers ", it seems worth while drawing attention to this anomaly.

The main derangement concerns the duodenal loop. In the adult fowl, the duodenum descends from the gizzard to lie slightly to the right of the midline of the floor of the abdominal cavity, in the pubic region it curls to the left bending sharply upon itself, and the two limbs of the U-shaped structure are closely

PLATE I



1. Liver.
2. Distended Gall bladder.
3. Pancreas in malpositioned duodenal loop.
4. Gizzard.

(Article by J. G. Carr, on page 234)

PLATE I

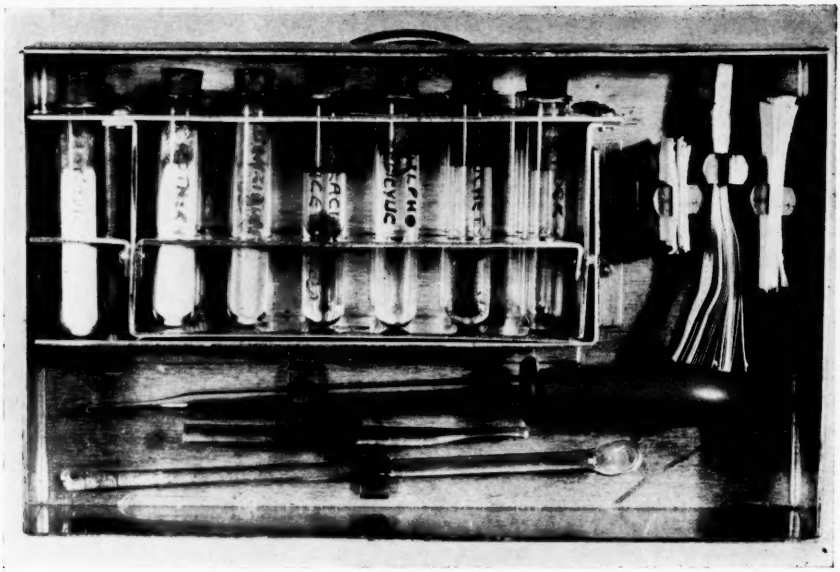


FIG. 1
Portable urine testing outfit.

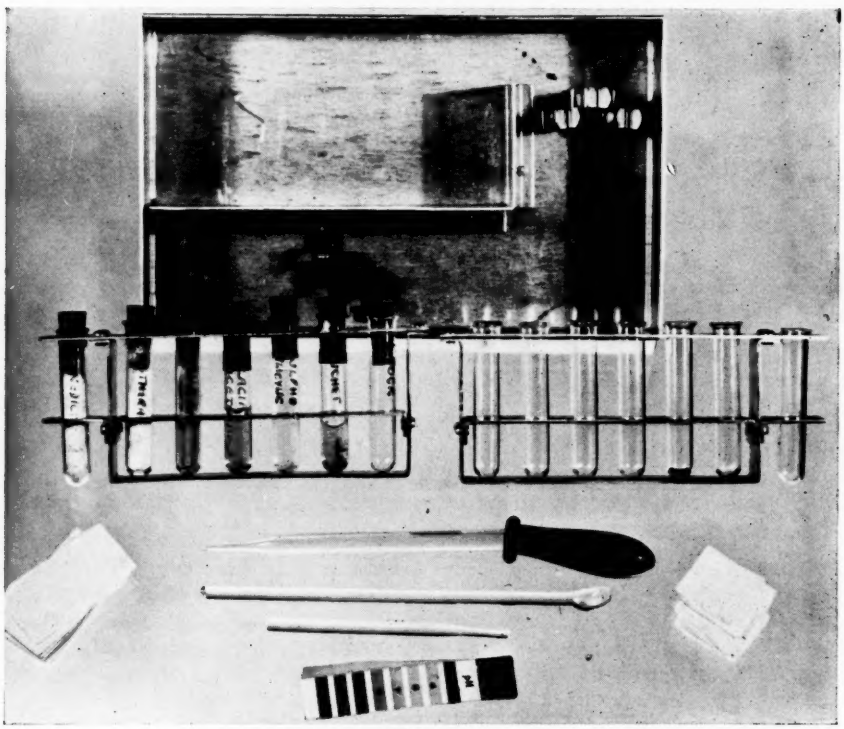


FIG. 2
Suggested order of chemical tests, excluding pH and Fouchet's.

(*Article by J. S. Wilkinson and D. G. Harvey, on page 238*)

apposed until the level of the gizzard is reached again. The pancreas lies between the two limbs of this loop.

In about 10 per cent of a series of birds of which a detailed record was kept, this arrangement was impaired. The loop was formed, but the lower half was reflected back upon itself, so that instead of the apex of the loop being in the pubic region it pointed towards, and often reached, the liver. Usually it lay on the ventral surface of the abdomen and to the right of the normally positioned portion of the loop. The point of reflexion could be anywhere along the loop. In one instance it was almost at the tip, so that only about 3 cm. of duodenum were involved. In this case the tortional forces, probably aided by the compression exerted by the rest of the abdominal contents, had caused a considerable degree of stenosis, and the bird was very thin and undersized when killed at four weeks of age. The maximal derangement can involve almost the whole of the loop, either upwards towards the liver or to the right to be compressed against the peritoneal wall. If it reaches the liver, usually the right lobe, the latter may be smaller than usual, obviously due to restricted space for growth, as its edge closely conforms to the surfaces of the displaced loop.

Though the displacement of the loop is the most evident abnormality seen on opening the bird, and probably the primary one, there was some displacement of the rest of the intestine in some birds. In these birds, the cæca did not lie curved underneath the site where the duodenum should lie, but were directed upwards or to the left. The central portion of the gut may also be disturbed, so that Meckel's diverticulum can be found only after a detailed search.

It is probable that serious malposition may cause impairment of the function of the abdominal air-sac. This point has not been studied by dissection, but it has been obvious that the birds with this defect exhibit considerable respiratory distress even after the relatively trivial disturbance of catching them in a cage. A few have been observed in such respiratory embarrassment that death seemed imminent.

Affected birds are usually backward in development and tend to avoid the rough and tumble life in a brooder. On a few occasions, they have been kept in small cages and survived until the age of about ten weeks. Despite the lack of competition for food, they continued to be small, backward and thin. The bird in the photograph was backward from the time of hatching, and died of " inanition " at the age of eight days. Apart from the intestinal derangement, and much wasting of the body, no other abnormality was found.

The cause of the abnormality is not known. Since the pancreas is reasonably normal, it can be placed as occurring later than the development of this organ. When dissected free, the loop retains its malformation. This is due to an alteration of the lengths of its respective sides, so that the reversed curve is the only possible one. What may be an early example of this abnormality has been noted in some dead-in-shell chicks which were examined at about the twentieth day. The duodenum should lie against the yolk sac so that further elongation together with the decrease in size of the sac will lead to the loop curling automatically in the correct direction in the pubic region. Occasionally the loop is found to be mal-placed already and this is due to its first limb being longer, not shorter, than the second, so that a reversal of the normal curvature is inevitable. This abnormality

has been found quite frequently in two of the lines maintained at this Centre, and in crosses between them, but absent in another. A genetical basis may therefore seem possible. The absence of reports of a similar condition from other workers suggests that this specific defect may be confined to the Centre flock, and this feature supports the theory of a genetical origin.

In general the problem of the "poor doer" has received rather scant attention from poultry pathologists. If other workers meet with abnormalities similar to those mentioned above the study of possible causes might be of great value. If the trouble should prove to be of a genetical origin appropriate measures could be taken to eliminate it. The problem is one which calls for the collaboration of anatomists, pathologists and breeders.

Summary

A malposition of the duodenal loop occurring in chickens is described. This condition, believed to be genetic in origin, resulted in poor growth of the affected birds.

All expenses in connection with this work were borne by the British Empire Cancer Campaign.

AN ATTEMPT TO DETECT DEMONSTRABLE ŒSTROGEN IN THE BLOOD OF THE PREGNANT BITCH

By A. E. HARROP, M.R.C.V.S.

Senior Lecturer, Department of Animal Husbandry, Royal Veterinary College, London

DURING recent years this department has received numerous enquiries as to whether it is possible to diagnose pregnancy in the bitch by biological methods, i.e. whether an increased concentration of either gonadotrophic or œstrogenic hormones can be demonstrated in the body fluids of the pregnant bitch.

Numerous workers have failed to demonstrate gonadotrophins in either the urine or the blood of the pregnant bitch (Cowie, 1948). For urinary œstrogens Cowie cites the results of several workers who either obtained negative results or œstrogens were present in such small quantity that they could not be used as the basis for a test of pregnancy. Cowie in his monograph (as. cit.) states that "the œstrogen content of the blood of the bitch during pregnancy does not appear to have been investigated".

The present investigation was undertaken to ascertain whether readily demonstrable œstrogens are present in the blood of pregnant bitches.

Source of Blood Samples

Blood was obtained from bitches which were proved to be pregnant at the time of sampling by the delivery later of a litter at full term. Thirty-one different

bitches were used. In the majority of cases only one blood sample was taken from each bitch.

The stages of pregnancy at which samples were taken were 12, 15, 18, 21, 23, 26, 29, 33, 37, 39, 43, 45, 48, 53 and 57 days respectively.

Approximately 10 ml. of blood was taken from the cephalic vein of each bitch. This was allowed to stand for four hours to allow for clotting and was then forwarded to the Royal Veterinary College by post, where the serum was drawn off and kept at 4° C. until required for injection. The total interval elapsing between withdrawal of blood from the bitch and the first injection of serum into the test animal was rarely more than 24 hours.

Technique

0.25 ml. of serum was injected subcutaneously twice daily for two days (total of 1 ml. of serum) into each of four oophorectomised female mice. (All the test mice used had been checked for sensitivity to œstrogen previously, and no mouse was used again until it had had four weeks' rest.)

On the third and subsequent days until the seventh from the commencement of injections, smears were made with a sterilised wire loop from the anterior vagina of each mouse at 12-hour intervals. The smears, after being dried and fixed in air, were stained by either the Giemsa or Leishman methods.

Results and Conclusions

All smears were negative, diagnosis being based on the fact that only very small numbers, if any at all, of cornified cells were observed on microscopic examination. It may be assumed, therefore, that œstrogen, if present at all, is not demonstrable in the blood of the pregnant bitch when the technique described above is used.

Acknowledgements

I desire to express my indebtedness to Messrs. S. L. Hignett, B.Sc., M.R.C.V.S., and J. Scarnell, B.V.Sc.(Liv.), M.R.C.V.S., of Burroughs Wellcome Veterinary Research Station, Frant, to S. J. Hodgman, M.R.C.V.S., Director of the Canine Research Station, Animal Health Trust, Kennett, Nr. Newmarket, and to Jas. K. Bateman, B.Sc., M.R.C.V.S., of the Greyhound Racing Association, for their kindness and co-operation in supplying the samples of pregnant bitches blood.

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ON THE ROUTINE CHEMICAL ANALYSIS OF SMALL VOLUMES OF URINE

ARTICLE II: A SIMPLE PORTABLE URINE TESTING OUTFIT

By J. S. WILKINSON and D. G. HARVEY

Royal Veterinary College, London

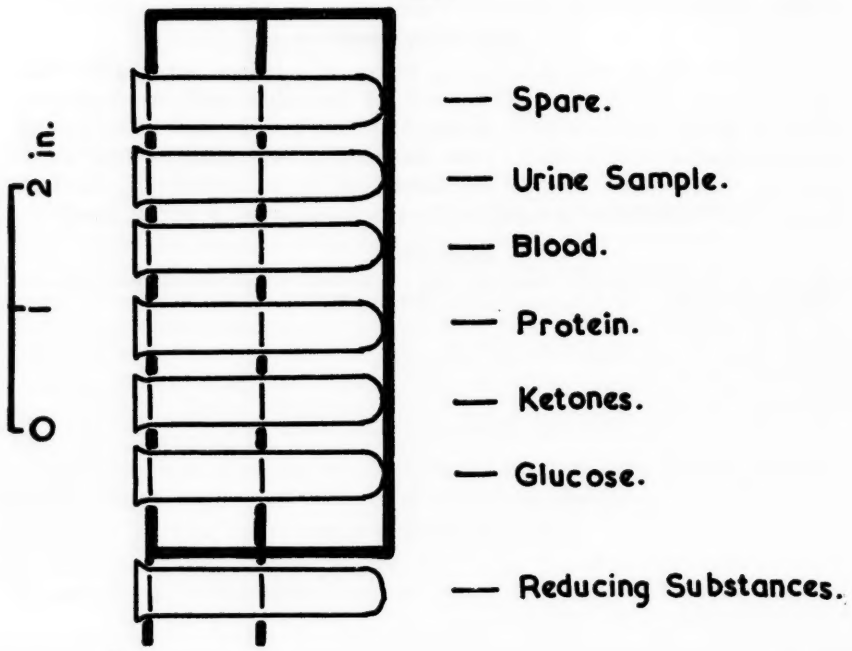
Introduction

In a previous communication, Harvey (1956) reviewed and appraised certain tests for the identification of abnormal constituents in small volumes of urine. Most of these tests can be performed with ease and some are suitable for inclusion in a small outfit for use in the field. A description is given of an outfit that contains all the necessary equipment and materials for performing these tests. The methods of preparing and using the reagents were set out in the previous paper. The tests are not necessarily in a final form but they have been tested many times on pathological urines and they have given results which are reliable and can be reproduced. One advantage of these "drop" tests is their economy. At present prices of AnalaR reagents, a few shillings will purchase sufficient chemicals (except Testape or Clinistix) and the simple equipment required to carry out nearly 1,000 tests for each of the six abnormal constituents which are found most frequently in urine.

Description of Outfit (Figs. 1 and 2)

A metal tin of "pocket" size is required. Flat or cylindrical tins can be adapted. The tin described measures 6¾ in. × 4 in. × 1 in. It is fitted with a lid,

TABLE I



an angular dividing wall and six small Terry clips. The dividing wall makes a compartment to house two small test tube racks. Each rack measures 5 in. × 2 in. × $\frac{5}{8}$ in. (Fig. 2) and it is sufficiently rigid to enable it to stand upright. Holes are drilled to accommodate seven rimmed 2 in. × $\frac{3}{8}$ in. test tubes. One set of holes is set outside the vertical strips of the framework in order that the rack can be used as a test tube holder when one performs a test requiring external heating.

Contents

In one rack there are seven rubber stoppered test tubes containing the reagents. Each tube contains enough dry reagent for about 15-20 tests without recharging.

1. Dry Benedict's Mixture for reducing substances. From a stock mixture of $CuSO_4 5H_2$, 1.7 g., Sodium Citrate 17.0 g., Anhyd. Sodium Carbonate 10.0 g.
2. Testape (proprietary enzyme test to identify glucose. Alternatively a set of Clinistix could be carried). Small pieces about $\frac{1}{4}$ in. square. This tube should be covered in a sheath of dark paper to protect the papers from undue exposure to light.
3. Rothera's Mixture for ketones. From a stock mixture of sodium nitroprusside 1 g., Anhyd. Sodium Carbonate 20 g., Ammonium Sulphate 20 g., very intimately mixed *but not* ground.
4. Sulphosalicylic acid for proteins. From a stock 5 per cent (w/v) solution in distilled water.
5. Benzidine Mixture for Blood. From a stock mixture of Benzidine Acetate 10 g., Barium Peroxide 20 g., Citric Acid 10 g.
6. Fouchet's Reagent for Bile Pigments. From a stock solution of Ferric Chloride, 10 ml. 10 per cent (w/v) in 100 ml. of a 20 per cent (w/v) solution of Trichloracetic acid.
7. Glacial Acetic Acid. 1-2 ml. (For development, if necessary, of the Benzidine Reaction—Harvey *loc. cit.*)

The other rack holds seven clean, dry test tubes. One of these should be fitted with a rubber bung as this tube is used for the storage of urine specimens.

Three of the Terry clips hold two small glass pipettes (Pasteur) fitted with rubber teats, and one short slightly tapered glass rod. The other clips hold 1in. × 1 in. filter paper squares previously saturated with 10 per cent Barium Chloride and dried, BDH Wide Range Indicator Papers (pH 2-10.5), some adhesive labels and small squares of filter paper.

In addition, the outfit should contain a piece of matt black paper to be used as a background for the protein test. A small phial of distilled water may be included in order to wash out the test tubes. While thorough tap water washing is quite adequate for cleaning the tubes after most of the tests, it is not satisfactory if the tube is to be used again for the Benzidine reaction. Alternatively, the Benzidine and Rothera's tests may be carried out on a small square of filter paper, on a glass slide or on a white tile. A few crystals of thymol in a small tube can be included so that specimens may be preserved for further examination.

Scope and Method of Using the Outfit

Five out of the seven tests involve the use of dry reagents. Of the other two,

only that for protein lends itself to a possible dry modification. A few milligrams of the sulphosalicylic acid can be carried in a test tube. So far no suitable substitute has been found for Fouchet's reagent.

A possible order of tests is suggested in Fig. 2. This, of course, excludes pH determination and Fouchet's test for which test tubes are not necessary.

No equipment or chemicals are included for specific gravity determinations. Details of two alternative determinations were given in the previous communication. A similar outfit for this determination can be built on the lines suggested for the chemical set described above.

Acknowledgements

We wish to acknowledge with thanks the valuable assistance of Mr. V. C. Tindley, in whose workshop the box and racks were made, and also Mr. A. R. Goffin for the photographs.

REFERENCE

Harvey, D. G. (1957): *Brit. Vet. J., 113,* 52.

REVIEWS

DIAGNOSTIC AIDS IN THE PRACTICE OF VETERINARY MEDICINE, by H. H. Bernier, B.S., D.V.M. Published by C. W. Alban & Company, St. Louis, Missouri. Pp. i-xi and 1-86. 6 in. × 9 in. Stiff cloth boards. $4.0

THE author is Associate Professor of Veterinary Pathology at the School of Veterinary Medicine, University of Missouri.

This pocket-sized book presents in a clear bold type the brief essentials of " simplified field laboratory procedures " which are used in the routine practice of the department of clinical pathology in the above veterinary school.

The text is arranged in tabulated or note form and brevity precludes the inclusion of any references. The first section gives a list of recommended books and charts and this is followed by a useful part listing details of diagnostic procedures with equipment and reagents needed. There are sections on hæmatology, the function of liver, pancreas, pituitary, hypothalamus and kidney, bacteriology stains and toxicology. Although the author recommends certain books on parasitology *no* parasitological techniques are described.

A full list of all the laboratory equipment required is given and much use is made of proprietary " standard " tests mostly in tablet form. Full details of the manufacturing companies with the current prices in dollars are appended. Many of the products are not available in this country. There is a useful index and a few blank pages are provided for the reader's own notes.

The author has exercised considerable ingenuity in presenting tests which are simple to perform. On the whole, with few exceptions, results are only qualitative or grossly quantitative. The margin of individual error in the visual assessment of many end-points described must be very considerable and this renders precise interpretation of many tests extremely difficult.

The work is a sincere attempt by the author to collect under one cover the main essentials of some of the standard laboratory tests and aids to diagnosis which

are based on a knowledge of clinical pathology. Unfortunately, this book does not cover adequately the needs of veterinary surgeons who practise in the British Isles.

STORIA DELLA VETERINARIA. Published by "Farmitalia," Servizio Veterinaria, Milan. September 1957.

"Farmitalia" is to heartily congratulated on the publication of this very excellent book dealing with the history of veterinary science. Profusely illustrated, with many coloured plates as well, and presented in a very pleasing *format*, the book is a delight in every way and is the sort of prestige publication that one would have hoped one of our own drug houses would have issued rather than wait as usual for this sort of thing to make its way to this country from across Europe.

As it is, we are indebted to "Farmitala" for an exceedingly able and intensely interesting review of the whole history of veterinary science in the very broadest since, beginning with man's earliest contact with animals in the far-off cave days, passing on through the dark and magic-bedevilled days of the Middle Ages and terminating with the dawn of true science, the foundation of the veterinary colleges in Europe and the institution of veterinary services. Everything is there from Cro-Magnon Man right through to penicillin. The book is rounded off by a brief account of the veterinary institutions of every country in the world together with an account of the eminent scientists who, at one time or another, have been members of their staffs.

This is a book that should be on every veterinary surgeon's bookshelf; that it is in Italian should deter no one from acquiring it as the language employed in the text is beautifully clear and simple and readily understood with the assistance of a little third-form Latin.

Copies of this book can be obtained only from the following address:—Farmitalia, Servizio Veterinaria—via Turati 18, Milano.

NOTICES

Third World Congress on Fertility and Sterility, Amsterdam, June 7 to 13, 1959

THE Third World Congress on Fertility and Sterility, sponsored by the International Fertility Association, will be held in Amsterdam, Holland, from June 7 to 13, 1959.

The general outlines of the sections of the programme will be as follows:

1. Female sterility (Physiology of reproduction, pathology, endocrinology, clinical problems, treatment).
2. Male sterility (Physiology of reproduction, pathology, endocrinology, clinical problems, treatment).
3. Basic research and/or animal reproduction.
4. Psycho-sexual problems.

Although any original report on some phase of fertility and infertility, either clinical or in the field of the basic sciences, will be considered, definite priority will be given to those papers concerning the following subjects:

1. Embryonic death (etiology, pathogenesis, placental structures in its relation

to the condition of the fœtus, functional problems and diagnosis of the embryonic and fœtal death, and habitual abortion).

2. Hormonal factors and vitamins in fertility and sterility. (Ovulation and sterility, induction of ovulation, influence of thyroid, steroids, etc. on ovulation, influence and physic-pathological significance of vitamins, hormones and spermatogenesis, vitamins and spermatogenesis).

3. Relative value of the techniques for study of the endocrine functions in human sterility (study of the estrogen function, study of the luteal function, study of the adeno-hypophysis function, testicular biopsy value, study " in vitro " of the fertilisation of mammalian and human ovums, use of radio-isotope designers in the study of the sexual function).

4. Biochemistry of spermatogenesis.

5. Psycho-sexual problems in sterility.

Official languages: English, French, German, Spanish (with simultaneous translation).

Registration fee: Full member U.S. $40; accompanying family-members U.S. $15; after January 1st, 1959, the registration fee for full members will be U.S. $50.

Titles for papers should be sent not later than June 1958 to both Dr. Alfonso Alvarez-Bravo, Avenida Horacio 1506, Mexico City 5, D.F., Mexico, chairman, and Professor Dr. B. S. ten Berge, Academisch Ziekenhuis, Groningen, Holland, executive member of the Programme Committee.

For further information and registration, apply to Dr. L. I. Swaab, Hon. Secretary, Third World Congress on Fertility and Sterility, Sint Agnietenstraat 4, Amsterdam-C, or to the nearest Wagons Lits/Cook office.

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